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Agricultural Experiment Station

OF THE

LOUISIANA STATE UNIVERSITY
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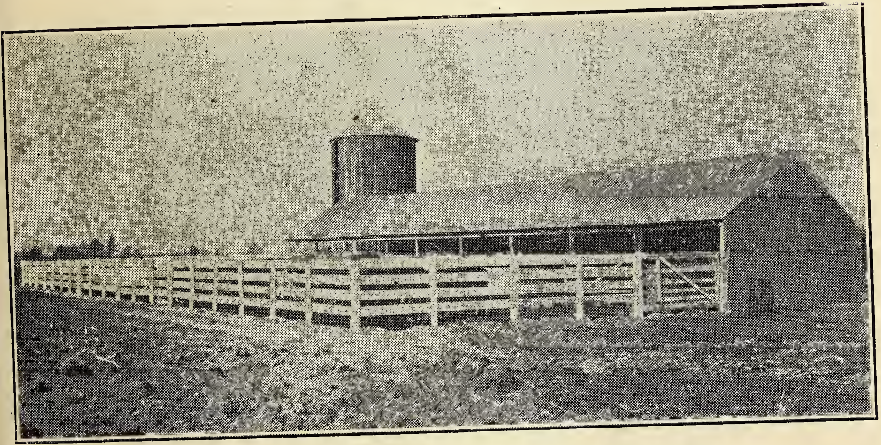
BATON ROUGE



Cattle-Feeding Equipment in East Baton Rouge Parish (Sheds, Pens, Silo and Scales). Comparatively Inexpensive

CATTLE FEEDING ON THE PLANTATION AND FARM

BY

W. H. DALRYMPLE, Veterinarian

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Cattle Feeding on the Plantation and Farm

By **W. H. DALRYMPLE**

(While the material in this bulletin was originally prepared for the information of the sugar planter, and presented as a paper at a meeting of the Louisiana Sugar Planters' Association, February, 1914, the data may, with certain modifications, be made applicable on other classes of agricultural holdings, and be helpful to those who may be thinking of taking up the branch of cattle feeding in connection with their other farming activities. —W. R. Dodson, Director.)

The idea of cattle as a side line on the sugar plantation is not altogether a new one.

Some years ago, during the days of our regular Farmers' Institute itineraries in the sugar belt, I remember suggesting the possibility of making profitable the institution of a cattle fattening department on the plantation during the grinding season, the idea being to purchase a sufficient number of selected steers in good feeding condition, and by the use of cotton seed meal as the chief concentrate, and all the green cane tops necessary as roughage, a large quantity of cane tops might be utilized to advantage, and a considerable "bunch" of good fat steers made ready for the market at reasonable cost at a time when a lot of such cheap, nutritious roughage was available.

Nothing seemed to result from the suggestion at the time, for the reason, doubtless, that conditions generally were more propitious with the planting industry, and the time not ripe for consideration of the project as it may now appear.

There was one large planter, however, who did seem to think the suggestion a reasonably feasible one, and made a purchase of some cattle with which to make a start, but then, as now, the cattle tick stood in the way of progress and success in the cattle business. Some of the cattle bought were not immune to tick fever, while others had fever ticks on them, the result being that the non-immune animals became infested with ticks from the ticky ones, the fever germs were transmitted to the susceptible cattle, and a number of them died from Texas or tick fever.

So far as my recollection serves me, the loss of cattle was a decided discouragement to the planter, and put a stop to the enterprise being further pursued on that particular place.

I am still of the opinion, however, that with proper management, a considerable number of butcher cattle might, even dur-

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ing the grinding season, be brought into marketable shape and leave a profit to the planter, under fairly reasonable market conditions.

Then, with suitable and convenient, though not necessarily expensive, sheds and lots in which to feed the cattle, the rich resultant fertilizing material might be saved for use on the land—no mean item in itself, when one considers that the voidings from the cow are estimated at 68 pounds in 24 hours, 49 pounds being represented in the solid excrement and 19 pounds in the urine or liquid manure.

Based upon the commercial prices of nitrogen, phosphoric acid and potash, viz., 18c, 5c and 5c, respectively, per pound, a ton of manure from the cow, and containing the following amounts of each of the fertilizing constituents—nitrogen, 8.5 pounds; phosphoric acid, 5.8 pounds; and potash, 8.8 pounds—is worth about $2.26.

Further, it is estimated that the yearly value of the manure from a cow, maintained under good conditions, is about as follows: Nitrogen, 171 pounds; phosphoric acid, 26 pounds; potash, 108 pounds, representing, in money value, $37.48.

I merely mention these figures to draw attention to the value of saving, for use on the land, the rich resultant manure from the feeding of cattle, more particularly steers, as the nitrogen content is greater in the excrement of the male animal than in that of the female, especially the milking cow, due to the fact that a certain amount of this element in the food is directed toward the formation of milk in that animal, but which largely appears in the manure of the male, such as the steer or bull.

In an intelligently conducted steer feeding department of this kind, then, there should not only be the profit on the increase of weight during 90 or 100 days, or so, but a large amount of a by-product, so to speak, rich in fertilizing value, which ought to be considered in the gross returns, and it might be further mentioned that the richer the feed, the richer the manure resulting from it—that is, in nitrogen, the most expensive element of plant food.

In this connection I may say that last year I had occasion to figure the manurial value of a ton of lespedeza hay, which is a leguminous forage, as compared to the same weight of timothy hay, which is a grass hay. Based upon the prices per pound for the three principal elements of plant food already mentioned, I found that the resultant manure from one ton of the lespedeza was worth approximately $9.46, while that from the timothy was worth only $5.13. I cite these figures merely to illustrate the fact that the manure from a feeding material which is rich in nitrogen is of greater fertilizing value than from one not so rich.

And while on this phase of the subject I might allude to another point as illustrative of a condition which frequently seems to prevail among those who dispose of most of the feeding material, such as hay, for example, they raise on their farms. The farmer is wont to boast of having sold all of his crop at a satisfactory figure, and views it as a wonderful achievement. If, on the other hand, the farmer should feed that hay to some good grade meat producing animals, such as steers, and send his hay to market "on the hoof," as the saying goes, he would not only receive a better price for it in the higher priced finished product, but in addition would have, as a gratuity, if you will, the manurial value of $9.46, in the case of lespedeza hay, for each ton the animals consumed. To dispose of feed crops off the farm, unless there be a surplus that cannot be profitably consumed, is one of the surest ways of selling fertility and depleting the soil.

As a general statement, and where diversified or mixed farming obtains, no feed crops should be sold off the farm in the raw state, but should be disposed of through the animal, after having been manufactured by it into a product of enhanced value.

So far I have only touched upon the use of the green tops during the grinding season, and while a vast amount of this roughage might be profitably utilized, it would not be practicable, perhaps, to make use of all of it in this way.

To enable the planter to store the bulk of this material for future use, the silo seems to fit in admirably.

Suffice it to say, that while a large number of cattle might be fed on green cane tops for roughage during the harvesting season, more continuous feeding of more animals could be accomplished by the use of the silo and the ensiled tops.

With regard to the cattle, and so far as I am able to judge at the present moment, there seems to be at least two ways in which to approach the project. One of these is to purchase the desired number of feeding cattle and finish them off for market, using the green tops, for roughage, during the harvesting season, as suggested, and where the silo had not yet been adopted, or to ensile the tops and store them, in which case more animals might be fed and the feeding continued through a longer period.

And the second method is to purchase breeding males of the beef type, some good selected native, or better, grade cows and heifers, and raise the feeders on the place. This would, necessarily, require a portion of the plantation to be set aside for this particular branch, and would involve a certain amount of risk to the animals during the time required to bring them to a suitable age for "topping off" for the market, but which would be considerably obviated if the cattle were bought ready to place in the feeding lots or pens. In some sections of the sugar belt, where charbon is liable to exist, cattle raised on the place would be more exposed to loss through infection during the period of their development than would those purchased and kept in lots during the relatively short fattening period, especially in the cooler season of the year.

I take it, of course, that all of you are aware of the presence of the cattle tick being the great drawback to the cattle industry in the State at the present time, not only as the carrier of the organism of Texas fever, but on account of its being the cause of the closing of the best markets of the country against our cattle in the quarantined area. However, it is to be hoped that in a very few years the tick will have been completely eradicated from our State, if only we could obtain more active co-operation in the work by our people.

That complete extermination of this expensive pest is possible is evidenced by the fact that, since 1906, the year that tick eradication in the South was begun, the total area cleared of ticks and released from Federal quarantine now aggregates about 220,000 square miles.

If the time had arrived when the State was tick-free, which would better enable our farmers to improve their grade of cattle, and be able to supply feeders to the plantations to be there finished off for the market, there is no reason why such an enterprise should not be mutually profitable to both planter and farmer.

Still, that time is not yet with us, and for the time being feeding cattle would have to be picked up wherever suitable stock were to be found. It should be added, however, that the better the quality of feeders, the better use they make of their feed; they mature more quickly, and are more profitable in every way.

In the selection of beef bulls for the purpose of grading, one is often asked as to which is the best breed. Between the three great modern breeds, viz., the Shorthorn, Aberdeen Angus and the Hereford, there is very little, if any, appreciable difference. They are all good, and all have been developed along similar lines by careful selection, breeding, feeding, etc., for the single purpose of producing high-grade animals for the butcher's block.

Each and all of these fine animals, however, require to be well fed from calfhood up, as careful feeding, and plenty of it, has played a large part in their splendid development.

Another question frequently asked is with reference to "rustling" qualities in the beef type of cattle. I do not believe that such a quality was ever considered, either by past or present breeders and developers of the modern beef animal, their aim rather being to produce a type of animal that would eat heartily, digest its food thoroughly, lay on flesh rapidly and get into marketable condition in the shortest possible time.

In the selection of breeding animals the first thing to consider is environment. Many people seem to permit their fancy in this matter to obscure their better judgment. Others, again, base their selection on the success of some one else whose conditions and environments may be totally different. Where there is always an abundance of nutritious food materials, however, any one of the beef breeds mentioned will most likely succeed under intelligent management. But where such is not forthcoming, none of these breeds is likely to prove a success. At the same time, with proper care and management, the large modern beef breeds make the most profitable butcher's animals, and it is the butcher who has the final say in the matter.

Whether the planter should decide to simply purchase good feeding cattle to finish off for the market—somewhat after the manner alluded to—or whether it should be his intention to breed and raise his own cattle to fatten, it will be imperative that experienced men be employed, who have been trained in the business, as it is an enterprise that requires as much intelligent, practical experience to make it a success as any other line of endeavor.

If properly conducted, I cannot see why this branch of industry should not be made to succeed from the very start. But in order to have it do so, it must be commenced under the most favorable auspices, especially with regard to those who are placed in charge of it. I am afraid, however, that we have very few men of this class, locally, at the present time, who could be entrusted with such an important undertaking. This is practically a new enterprise with us, and very few, if any, of our people know anything about it. Hitherto the live stock of the farm have been treated more or less as outcasts, with very little or no attention paid to their proper care and development. They have been allowed to struggle along as best they could, under adverse conditions, or die in the attempt, and the remaining ones represent, more or less, the survival of the fittest. Such a state of affairs cannot possibly be permitted to exist if we expect to make profitable the business of preparing cattle for the best beef marts of the country.

From the day the calf is born until the day when the matured animal is consigned to the butcher, the rule to be observed should be: Continuous progression, and no retrogression.

One authority has made the statement that "half the disease, death, and mismanagement amongst animals, and especially cattle, is traceable to insufficient and incompetent attendance.''

And another, in describing the qualifications of a good cattleman, says: ''A good cattleman makes his business a hobby, takes pleasure in his work, studies the comfort, likes and dislikes of each animal under his charge, and does for them, of his own accord, what no amount of instruction or supervision could insure. He readily detects when anything is amiss with an animal, and he knows when to give, and when to withhold, when the food should be increased, and when reduced, etc.''

Men of this class are what are needed in the business of either raising cattle or of fattening them for the market with the greatest assurance of success. No doubt they are to be had; in fact, I feel reasonably certain that they can be secured, but not locally. They will have to come from other sections of the country for the present, at least, where they have had the practical experience in just this sort of work.

Harking back for a moment to the manurial value of feeding stuffs and more particularly cotton seed meal, I may say that some time ago I had referred to me an inquiry from a correspondent of the Breeder's Gazette, of Chicago, regarding the value of this meal as a fertilizer after it had passed through the digestive tract of a steer or a cow. The data which I obtained is quite interesting, and I thought it might be worth while reciting in connection with our subject.

In order to secure the information, I took occasion to refer to some of the classic work of Lawes & Gilbert at the Rothamsted Experiment Station in England. The work of these scientists in this connection was based upon decorticated cotton cake, which, however, would represent a good quality of cotton seed meal.

In one ton of 2,240 pounds of this feeding stuff there are, approximately, the following amounts of fertilizing elements:

Nitrogen	147.84 pounds
Phosphoric acid	69.44 pounds
Potash	48.00 pounds

In the case of the milch cow, giving about 10 quarts of milk daily, the total of each of these fertilizing ingredients remaining in the manure, after the amounts in the milk had been deducted, were as follows:

Nitrogen	128.57 pounds
Phosphoric acid	62.26 pounds
Potash	38.58 pounds

In the manure from the steer, the following were the amounts:

Nitrogen	143.46 pounds
Phosphoric acid	66.48 pounds
Potash	44.42 pounds

It will be noticed here, as previously alluded to, that the manure from the steer contains a greater amount of the elements of fertility than that from the milch cow, due, as I have already stated, to a certain amount being diverted in the milk.

Basing our calculation upon the long ton of 2,240 pounds, and using round numbers only, and at the following prices per pound for the different ingredients, viz., 16c for nitrogen and 5c each for phosphoric acid and potash, we obtain the following approximations:

Manure from the milch cow from 2,240 pounds of a good cotton seed meal:

128 lbs. nitrogen, at 16c	$20.48
62 lbs. phosphoric acid, at 5c	3.10
38 lbs. potash, at 5c	1.90
Total	$25.45

Manure from the steer from 2,240 pounds of a similar meal:

143 lbs. nitrogen, at 16c	$22.88
66 lbs. phosphoric acid, at 5c	3.30
44 lbs. potash, at 5c	2.20
Total	$28.38

Reducing the foregoing figures from the long ton of 2,240 pounds to the short ton of 2,000 pounds, we find the fertilizing value of the manure from the milch cow to be $22.68, and that from the steer $25.26.

While the calculation may not work out in practice just exactly as here shown, it will at least afford an approximate conception of the relative value of cotton seed meal in the manure from the milch cow and from the steer—and especially the steer in our case—fed this valuable feeding stuff.

What I am desirous of emphasizing in this connection is the large amounts of fertilizing elements, orignally in the feed, that may be recovered in the manure, and when properly saved and carefully utilized, is one of the chief sources of profit accruing from steer feeding operations. Or, in other words, the feeder secures two values, viz., that from the fattening increase, which ought to more than pay for the feed, and that from the manure, which is almost equal in value to the original cost of the feed.

Through the courtesy of Mr. W. P. Connell, of East Baton Rouge Parish, I have been able to obtain some quite interesting data in connection with steer feeding, which he undertook last winter.—1912-1913.

Mr. Connell's chief purpose in inaugurating this work was to secure from the operation some rich fertilizing material to incorporate with his farm land, add vegetable matter to his soil and increase the fertility for future plant growth.

The steers purchased were of the native variety, in the main, although selected to some extent, with a few beef grades and one or two grades of the Brahmin or Zebu breed.

Had all of the steers been good beef grade animals, while costing a little more at the beginning, the ultimate results would, no doubt, have proved more satisfactory.

Incidentally it might be mentioned here that the beef grades were the first to be picked out by the buyer (in the New Orleans market), and brought the highest price, while the Brahmin grades, although the largest of the shipment, were the last to be looked at—the explanation given being that they did not cut up so profitably on the block.

The number of steers fed on this occasion was 195 head, and the feeding lasted for 90 days.

The gross weight of the 195 head when purchased was 127,870 pounds, or an average of 666 pounds per head.

The price paid per pound was 4 cents, which amounted to $5,195.80.

The total gain in 90 days was 37,620 pounds, or an average daily gain per head of 2.14 pounds.

The gross weight when sold was 167,515 pounds, which realized the total of $11,745.78, or a general average of a fraction over 7 cents per pound.

The average cost per one pound of gain was 10.29 cents.

The net profit per head was $13.74.

The net profit on the 195 head was $2,680.86.

FEED AND COSTS.

Cotton seed hulls at $9.00 per ton	$1,783.10
Cotton seed meal at $27.00 per ton	1,235.38
Molasses at 6 cents per gallon	420.00
Labor	308.80
Interest	82.34
Other expenses	39.50
Total	$3,869.12
Gross returns from cattle	11,745.78
Cost of cattle and expenses	9,064.92
Net profit	$2,680.86

The manure from the 195 head in 90 days, Mr. Connell reckoned at three-fourths the value of the original cost of the cotton seed meal, which is not an unreasonable estimate.

The crop increase in corn from the land on which the manure was placed was ~~62.5~~ 60 per cent., or the difference between 40 bushels per acre and 25 bushels per acre where the manure was not used.

This year Mr. Connell is feeding 215 head of steers of rather better grade. They cost him 4¾ cents per pound. He is feeding cotton seed meal at $30.00 per ton, molasses at 7½ cents per gallon and corn silage which he estimates is costing him $3.50 per ton.

Out of a part of last year's profit he has built a $1,000.00 silo—63 feet high by 20 feet in diameter—and has filled it with corn, chiefly made on land fertilized with the manure from the previous winter's feeding operations.

The present lot of steers are still on feed, and so far have gained an average of 2.1 pounds per head per day.

His method of using the meal for the size and weight of the cattle he is feeding has been to allow two pounds per head per day for the first 30 days, four pounds for the next 30 days, and six pounds for the last 30, making 90 days in all.

Mr. Connell's cattle have always been dipped and tick-free when placed in his feeding sheds.

It is, of course, difficult to say just how this winter's feeding may turn out, when the cattle are ready to ship. However, should the market be reasonably good at the time, the chances are that the results will prove satisfactory.

Since this paper was originally prepared the writer has been able to secure some figures from Mr. Connell concerning a bunch of 60 head of those just referred to, and which will no doubt be of further interest on this subject. The feeding period was 111 days and the materials used were cotton seed meal, blackstrap (cane) molasses and corn silage.

The folowing is the financial statement furnished by Mr. Connell:

Total sales, 60 head	$3,870.16
Purchase price	1,999.93
Gross profit	$1,870.23

Expense Account.

Silage, 165 tons at $3.50	$577.50	
Molasses, 79 barrels at $3.50	276.50	
C. S. Meal, 321 sacks at $29.50 per ton	473.37	
Labor, 111 days, at $1.00	111.00	
Interest on notes	40.34	$1,478.71
Net profit		$ 391.52
Net profit per head		6.52

Cost of feed per head, $24.65.
Cost of feed per head per day, 00.22.2c.

The above feeding operations were conducted during the winter of 1913-1914.

The silage above charged includes 30 tons of damaged material, which was not fed, and which would amount to $1.75 per head, or 00.1.58c per head per day, which should be deducted from the expense account. It should be stated also that the labor charge would have cared for 120 steers.

The average amount of each of the materials fed per head per day was as follows:

Silage	49 pounds
Molasses	6 pounds
Cotton seed meal	5 pounds

As I have mentioned that Mr. Connell was using corn silage, it may be of interest here to give the composition on the average of both corn and cane top silage:

Corn Silage.

Protein	1.70%
Carbohydrates	11.00%
Fat	0.80%
Fiber	6.00%
Water	79.10%
Ash	1.40%
	100.00%

Cane Top Silage.

Protein	1.25%
Carbohydrates	11.83%
Fat	0.44%
Fiber	8.04%
Water	76.50%
Ash	1.94%
	100.00%

Some more recent analyses give corn silage a slightly higher protein and carbohydrate content. However, from the above showing it would seem that, for all practical purposes as a roughage, corn silage and cane top silage would be almost interchangeable.

My purpose in giving the results of Mr. Connell's work, and there is at least one other feeder in East Baton Rouge Parish, Mr. K. H. Knox, who is making similar headway in this industry, was to afford you some idea of the practical side of the question in the State, and which I trust may be helpful to you.

I may be sowewhat off in my judgment concerning this matter, but when we take into consideration that the great cattle ranges of the West are rapidly being placed under cultivation in order to meet the demands of an ever-increasing population, both natural and through immigration, who have to be provided with occupations, and whose stomachs have to be filled, and with a shortage of beef cattle all over the country at present, and likely to be for some time to come, and with such an abundance of cheap materials available that might be turned into good marketable beef, I am at a loss to see why the present or near future should not be most opportune and propitious for our plantations to take up as a side line of considerable dimensions and make profitable, both directly and indirectly, the feeding of beef cattle.

The chief difficulties at the present time, so far as I can see, are:

(1) To be able to secure a desirable grade of profitable feeding cattle for those who should decide to feed only.

(2) The present scarcity, locally, of men who know anything about the business; and

(3) The presence of the cattle tick, which prevents freedom of movement of finished cattle to the best markets of the country.

When pursuing an enterprise of this character it is necessary that the best markets should be accessible at all times whenever the product was ready to be disposed of. This condition can only be brought about when the tick is destroyed and the section or parish is released from Federal quarantine.

Not only does the presence of the cattle tick close the most desirable markets in the North and West against shipments of our beef animals, through quarantine, but it renders risky the importation of susceptible breeding cattle from above the quarantine line on account of Texas fever, the germ of which is transmitted by the tick to non-immune stock, so that this parasite is an immense hindrance to our progress in this line of industry, both "coming and going."

However, with a good grade of animals, with no end of inexpensive feed in the main, with suitable and experienced cattlemen to intelligently superintend the work, with the cattle tick exterminated, which is a very simple process with the right kind of co-operation, and with the great beef markets ready and waiting to be supplied with good material, I repeat, I cannot at present see any good reason why cattle feeding should not become quite general on our plantations and become in time a very extensive industry and one of the great sources of supply, not only for local markets, but those of greater prominence throughout the North and West.

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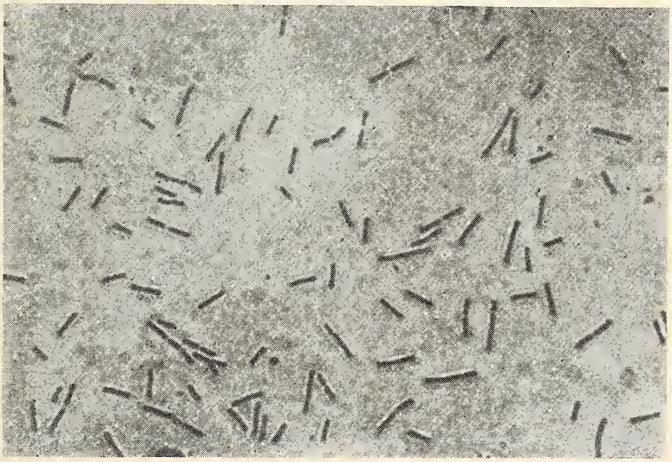


Anthrax bacilli from blood smear.

ANTHRAX OR CHARBON

Points of Popular Interest

BY

HARRY MORRIS

Bacteriologist and Assistant Veterinarian.

ANTHRAX OR CHARBON.

POINTS OF POPULAR INTEREST.

By Harry Morris.

This bulletin is designed to answer in brief form, free from technical expressions, all the important questions that come up in the discussion of the control and eradication of charbon or anthrax.

What is charbon or anthrax? Charbon is an acute infectious disease of man and animals.

History. One of the oldest diseases known to medical science.

Distribution. Almost world wide, much more common in Europe and Asia than in America. In the United States charbon is found in several states, but most commonly in the Lower Mississippi Valley and along the Gulf of Mexico.

What is the cause of charbon? A very small germ or organism known as Bacterium anthracis.

What is a spore? The spore of a germ or bacterium may be likened in a certain way to the seed of a higher plant. Keep the seed in a cool dry place and it may be kept for years, but supply heat and moisture and germination soon follows. Spores will live for years in soil or water without losing their power of germination. The spore is much more difficult to destroy than the rod in which it forms, just as is the seed of a higher plant.

How does the land become inoculated with charbon germs? From the carcasses of infected animals and also from the excrement of infected living animals.

How long will the germs live in the ground? For a number of years under favorable conditions.

What are the forms of charbon? External and internal.

What are the symptoms of the external form? This form is shown by a localized watery swelling at the point of inoculation. This swelling is hot and painful at first but afterwards becomes cold and apparently painless. It will soon spread under the skin along the course of the absorbent vessels. This form is usually seen in horses and mules and may occur in man as malignant pustule or carbuncular anthrax.

What are the symptoms of the internal form? Unless the animal is under very close observation no visible symptoms are noticed, death is very sudden and without apparent cause. The animal is seized very suddenly with great depression and prostration; rapid rise in temperature and fall of same just before death, heart action is rapid; mucous membranus of eyes, nose and mouth of a dark red color; solid and liquid (urine) excrements may have a blood-stained appearance. As this form of charbon is found most commonly in cattle, mistakes are often made in identifying the disease; the hides are removed and the carcasses are not destroyed as they should be. Under no conditions should the hide of an animal be removed during an outbreak of charbon without first making a microscopic blood test for the charbon germs. Skinning a carcass not only spreads infection but endangers the life of the person removing the hide. Many people hold the false opinion that every case of charbon must show an external swelling. Sudden deaths of animals during the summer months should be considered suspicious of charbon unless the cause of death is absolutely known.

How is the first animal infected? The first case of the season is usually one of internal charbon caused by eating or drinking food or water infected with the germs.

How do outbreaks of charbon occur? Failure to observe the first cases and neglect to properly (sanitarily) dispose of their carcasses are important factors in the history of outbreaks. The following examples have been selected as typical illustrations of the above point.

Case No. 1. Dairy herd; cow died suddenly, supposedly from the effects of a fall, broken neck; skin was removed and carcass buried carelessly: Subsequent developments indicated this to have been charbon. Result: Outbreak of charbon with a loss of thirty head.

Case No. 2. Stock farm; bull found dead; cause of death diagnosed as ''bloat.'' Skin was removed and the carcass dragged to the swamp and left exposed. Result: Loss of twenty-five head.

Case No. 3. Hides were removed from several animals found dead in the fields and cured with salt. Later they were sold, delivered to the local station and thrown upon the freight platform, where they remained for some time. Teams licked the hides for the salt and at the same time infected themselvs with the germs of charbon. Result: Outbreak of charbon.

Case No. 4. A swelling was noticed on the breast of a horse. The animal was taken to a secluded spot on the farm and the swelling covered with burlap sacks soaked in creolin. Death occurred during the night and the carcass was cremated at daybreak or before the flies had an opportunity to feed. A blood smear was examined by the writer and diagnosed as charbon. Result: The only case.

Case No. 5. Stock farm; five hundred head of cattle. Cow found dead in the pasture. Before burning a pound of gunpowder was sprinkled around and over the carcass and touched off with a fuse. Practically all of the flies were destroyed by the explosion. A blood smear taken from the animal was examined by the writer and diagnosed as charbon. Result: No more deaths on the farm.

The above cases illustrate some of the many that are reported indicating that lack of sanitary measures in disposing of sporadic (scattered) cases may bring about a general outbreak.

Do flies transmit charbon? Yes. In this laboratory we have been able to transmit charbon from infected guinea pigs to healthy ones through the bite of the horn fly.

How can the fly be controlled? In the external form of charbon cover the swelling with burlap sacks and keep them saturated with a good disinfectant or fish oil as a repellant to flies.

While preparations are being made for burning, the carcass should be covered with a good disinfectant or better with a piece of canvass that can be destroyed with the carcass.

The powder experiment noted in Case No. 5 above might prove successful. The most important points in the control of the fly is the rapid and complete destruction of all carcasses.

How should a carcass be destroyed? Burn immediately on the spot if practicable by placing the carcass in a gridiron made of green poles or iron rods, and the fire built directly under it so that burning can be effected by using the least amount of fuel, either wood or crude oil. If necessary to remove the carcass for disposal plug all the natural openings of the body with cotton or rags saturated with some disinfectant to prevent the discharge of infected material.

If burning is impossible bury the carcass in a hole six feet deep and spread lime thickly both above and below it. Bury the blood-saturated soil in the hole. After the grave is filled heat all the shovels by passing through a flame to kill the germs of charbon that might be on them.

A good burial is better than a poor burning.

How can the ground around a grave be sterilized? By burning the area over with crude oil or straw or by covering with lime and a good disinfectant, such as crude carbolic acid or some of the coal-tar dips on the market.

How are the germs of charbon diagnosed? By a microscopic examination of the blood; inoculations of the blood into guinea pigs and recovering the germs from their blood, and by growing the germs outside the animal body.

Can the germs be found in the blood of the living animals? Not usually except for a very short time before death.

How should a specimen be prepared for examination? Prepare two pieces of clean window glass about one inch wide and two inches long. Place one drop of blood toward the end of one piece of glass and with the small edge of another piece spread the drop out into a thin smear, then dry thoroughly in the sun or air. Prepare a second specimen in the same manner, place the two smeared surfaces together, pack securely and mail to the Louisiana State Live Stock Sanitary Board, Baton Rouge. The address of the party sending should be carefully indicated on the package and an explanatory letter sent to the Board.

The blood can best be secured by puncturing the vein at the base of the ear with some sharp steel instrument, which should be boiled for ten minutes if it is to be used again for any purpose.

Why send two pieces of glass? One blood smear is stained and examined with the microscope. If unable to make a diagnosis in this manner, the other is used for making cultures and inoculation tests.

Why should the blood be dried in the sun? To prevent the growth of other germs in the blood.

Why is it not advisable to send blood in a bottle?

1. It is contrary to the United States postal laws to send dangerous material of such character.
2. In this warm climate the blood is decomposed before it reaches the laboratory and is worthless for examination.
3. Infection is spread while collecting the blood.
4. The flies are afforded a better opportunity to fill themselves with the germs.
5. The lives of the collector, carrier and bacteriologist are endangered.

Is the milk from a cow sick with charbon dangerous for human use?

Prominent investigators in different parts of the world have not found such milk to contain the germs of charbon and our own investigation seems to have verified these conclusions. Although we do not advise the use of milk from an animal showing signs of charbon sickness during an outbreak.

Can charbon be cured? In the case of a small swelling resulting from direct inoculation of the virus (germs) it is possible, by the injection of a sufficiently strong germicidal solution (such as 5% solution of pure carbolic acid) directly through the skin into the swelling underneath, to destroy the local infection at that point. To prevent spreading of the swelling this should be done as early as possible. There are other swellings which may occur later that are not from direct inoculation but are secondary enlargements. In such a case the above suggested treatment would not be so effective. Besides, many animals recover after showing those secondary swellings.

During the latter part of an outbreak the disease is not so acute and many animals recover in spite of, rather than, the result of treatment. In such cases the treatment is often erroneously given credit for the cure. All over the world the control and eradication by strict sanitary measures is considered of much greater importance than the treatment of individual cases.

Should swellings be opened? No. The shedding of blood by the opening of swellings or through any other means is one of the surest ways of spreading charbon infection, as the blood or discharge contains the germs.

If any should fall on the ground the latter should be thoroughly disinfected.

Is vaccination a sure preventive measure? While a positive answer can not be given to this question, as there is no preventive measure of any kind that is absolutely sure, it may be stated that the vaccination against charbon is the best preventive measure known, provided the material is perfectly reliable; that it is used at the proper time and that the necessary antiseptic precautions are adopted in its administration.

During the past four years the writer has vaccinated, annually, the live stock on the Experiment Station farms, numbering about one hundred head, and, although infection of charbon has existed on the pasture, during the above period we have only lost one animal from the disease. However, all the necessary precautions were adopted.

Of what does vaccine consist? Live germs of charbon that have been attenuated or weakened. Dose No. 1 of the double dose vaccine is weaker than Dose No. 2.

Will vaccination produce charbon? To a limited number of highly susceptible animals it may; the percentage of loss through vaccination, however, is very small and should not deter the free use of the vaccine. If any animals should die the carcasses should be promptly disposed of.

When should stock be vaccinated? Begin vaccination not later than the middle of February or the first of March.

What stock should be vaccinated? Cattle, horses and mules, although smaller animals should be kept from sources of infection.

How should the vaccine be injected? Boil the syringe for ten minutes; after washing the point of inoculation with a 5 per cent solution of carbolic acid, inject one cubic centimeter or 15 drops (directions usually accompany the package) of the vaccine under the skin.

In cattle the injections are best made just behind the shoulder; we usually make the first injection on one side, the second upon the opposite side. In order to prevent collar pressure upon the point of inoculation horses and mules should be inoculated under the skin on the side of the neck.

Vaccine No. 2 should be given ten days after No. 1 and in the same manner.

DON'TS ON VACCINATION.

Don't give vaccine No. 2 unless No. 1 has been given.

Don't open the package until you are ready to vaccinate.

Don't use vaccine if the cork has been removed for any length of time.

Don't wait until an outbreak of charbon before vaccinating.

Don't use a dirty syringe.

After vaccinating burn all empty bottles and boil the syringe again.

How soon after vaccination is protection afforded? It is generally claimed that about thirty days are required from the first injection to give protection.

How long will this protection last? From six months to one year is the usual claim.

Can milk be used during the period of vaccination? We have never known of any ill effects.

Can horses and mules be used during vaccination?. Yes.

Is it advisable to vaccinate twice a year? In the infected areas of the state some vaccinate in the early spring and late fall with satisfactory results.

Should vaccination be practiced during an outbreak of charbon? Yes, if the animals are protected from infection during the time (one month) necessary to bring about immunity. They should be prevented from grazing on infected pastures or drinking at suspicious watering places, and should be protected from horse flies and other insect pests and every other necessary precaution taken.

There is a certain risk from vaccination during an outbreak unless the above precautions are adopted.

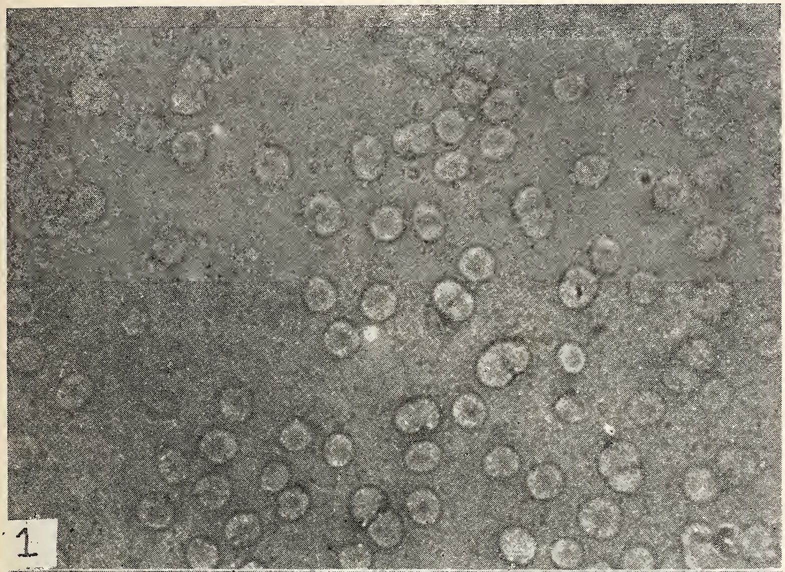
What measures may be necessary to clean a farm of infection? In addition to the protection of the animals and general sanitary measures, it is considered necessary in order to eradicate the infection from the surface of the ground to drain and cultivate infected lands.

Care should also be taken in the feeding of crops grown on lands known to be infected with charbon, as outbreaks have been recorded as occurring from the use of such feeding materials.

Do carrion feeders spread charbon infection? Without doubt the carrion feeders play an important part in the continuance and spread of charbon in Louisiana. The buzzard or carrion crow is the most important because it is capable of carrying infection for many miles after feeding upon an anthrax carcass, and may start fresh centers of infection in pastures where it has never previously existed. The germs are carried on the feet, beak, and in the vomitus of the birds, but not in the excrement.

The germs of charbon may be found in the excrement of the dog, hog, cat and opossum for many days after eating flesh from an anthrax carcass. A detailed report on this subject is given in Louisiana Bulletin 136.

Neglect to properly dispose of anthrax carcasses is, wihout doubt, the factor most responsible for the continuance and spread of anthrax in Louisiana.



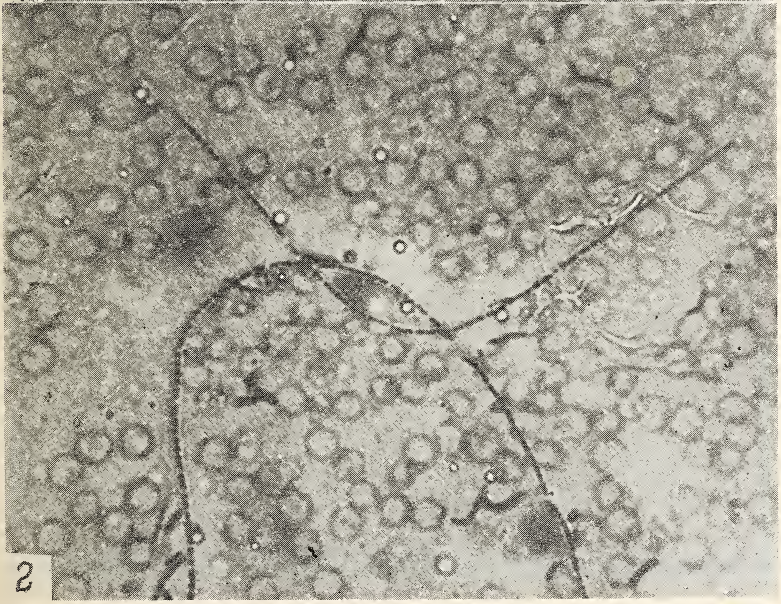


PLATE I, Fig. 1. Picture of blood from a healthy animal, showing the blood cells. Enlarged 700 times.

Fig. 2. Picture of blood from an anthrax animal, showing blood cells and chains of anthrax germs. Enlarged 700 times.

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Baton Rouge

The Comparative Value of Various Germicides for Use in Cane Sugar Factories.

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THE COMPARATIVE VALUE OF VARIOUS GERMICIDES FOR USE IN CANE SUGAR FACTORIES.

INTRODUCTION.

It is quite probable that the practice of using some form of germicide for preventing the growth of microorganisms in sugar factories existed at an early stage of the development of the sugar industry. This practice today, just as it was at a much earlier period, is almost entirely lacking in uniformity, and may be regarded as a purely empirical process. The use of formaldehyde as a preservative of juices in cases of emergency is, on the contrary, based upon the results of special investigation and is a thoroughly efficient procedure. But when it becomes necessary to outline a systematic campaign for cleaning sugar factories no data is obtainable upon the kind and amount of germicide that would prove most efficient under these conditions. So it frequently happens that whether formaldehyde or milk of lime, or some other germicide is used for this purpose, depends more upon the convenience of their respective use than their relative efficiency under the given conditions. Such haphazard methods could scarcely be expected to prove efficient, for should they happen to be effective in any instance it would probably be at an excessive cost.

There has recently been an awakening of interest among sugar manufacturers, in the importance of maintaining cleanlier conditions in the sugar factory. The importance of manufacturing raw sugars that can be kept in storage without deterioration has been fully impressed upon the manufacturers by the losses which they have frequently incurred from this source. That sugar deterioration is in a large measure due to the lack of care in maintaining the proper conditions in the factory is now so fully appreciated that few manufacturers are indifferent towards proposals for correcting these conditions. A question perhaps unheard of in the sugar industry ten years ago, which is now frequently asked, is: "What is the most economical and efficient germicide for sugar factory use?" To give this question a definite and authoritative answer has naturally necessitated a special investigation of the efficiency of germicides under sugar factory conditions. The reputed efficiency of most germicides has been derived from investigations of their action upon pathogenic species of bacteria, under conditions pertaining to pathology. Germicides vary in their action, not only with the species upon which they are tested, but also with the composition of the substance with which they must be brought in contact to perform their work. It is obvious therefore that we could not accept unreservedly the reputed values of these substances, should we attempt to apply them to sugar house conditions. The present investigation, the results of which are given in the following pages, is an attempt to standardize the present use of germicides in connection with the cleaning of sugar factories.

The thanks of the author is due Mr. Taggart, whose kind assistance, not only in the chemical analyses required in the investigation, but also by many helpful suggestions, greatly facilitated the progress of the work. To Mr. Inami, who, in the capacity of assistant during the latter stages of the investigation, rendered very faithful service, the writer also wishes to express his thanks. For the contribution by the Perth Amboy Co., of New York,, through their local agent, Mr. Bodenbender, of the formaldehyde used in the investigation, due thanks are here given.

Chapter I.

PLAN OF THE INVESTIGATION.

SELECTION OF GERMICIDES.

For a germicide to be suitable for use in sugar factories it must conform to the following standards: (1) It must be cheap. (2) It must be easily obtainable. (3) It must be practically nontoxic to the human system, or if slightly toxic it must admit of being easily and completely removed from the surroundings in which it is used, so that it may be employed without danger. (4) It must possess a relatively high germicidal action. (5) Its germicidal action must be prompt as well as effective. With the exception probably of the last of these conditions their fulfillment by a germicide will appear obviously essential for sugar house use. Upon reflection it will appear equally essential that the germicide perform its work within a comparatively short period of time. In making a selection of germicides for sugar house use we are necessarily confined within a rather narrow range. The restrictions are greater than in the field of pathology, on account of the larger amounts of substance to be used, the greater complexity of conditions, and the much lower limit of price, beyond which their use would prove prohibitive.

The following germicides were selected for the investigation: formaldehyde, milk of lime, chloride of lime, sodium fluoride, ammonium fluoride, sodium bisulphite, and calcium bisulphite. Their selection, as well as the proportions in which they were used, was based upon the manner in which they have been employed either in the sugar industry or in other somewhat closely allied fields of work. The germicides were employed in the following strengths:

Formaldehyde	0.5%
Chloride of lime..................	2.0%
Ammonium fluoride	5.0%
Sodium fluoride	5.0%
Milk of lime.....................	5.0%
Bisulphite of lime...............	5.0%
Bilsulphite of soda..............	5.0%

Some of the uses of these germicides in the sugar industry, and in other closely related industries, are given as follows:

Formaldehyde.

Jorgensen ([1]) recommends the use of formaldehyde in 0.5 per cent strength, for use in breweries and distilleries, for the cleaning of vats. Its use in the beet sugar industry for the prevention of fermentation in the diffusion batteries has been recommended by O. Friedrichs ([2]), who secured a patent for its use in this connection. He recommends its use in the proportion of 2½ to 5 grams to 100 kilos of beets. A similar use for formaldehyde has been recommended by Schulz ([3]), F. Strohmer ([4]) and M. Gonnermann ([5]) and others.

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(1) Microorganisms and Fermentation, p. 32.
(2) Lafar Technische Mykologie, p. 460.
(3) Deutsche Zucker Industrie, 1903. Bd. 28 S. 2017.
(4) Oestereich Ungarische Zeitschrift, 1905. Bd. 34 S. 685.
(5) Deutsche Zuckerindustrie, 1905. Bd. 30, 145-185.

Kamerling ([1]) recommended the use of very dilute solutions of formaldehyde in treating raw sugars to prevent their deterioration. The principal use of formaldehyde in the cane sugar industry at the present time is for the preservation of juices or syrups in emergencies, and for the preservation of laboratory samples. For the preservation of clarified juice Spencer ([2]) recommends the use of formaldehyde in the proportion of 3 cc. of formalin to a cubic foot of juice. In connection with the preservation of laboratory samples with formaldehyde, it is interesting to note the work of Norris ([3]) on the changes in the polarization of sugar solutions due to the addition of formaldehyde. Simpson ([4]) recommends the use of formaldehyde on all mill juice to prevent fermentation in the factory. He recommends its use in the proportion of one part of formaldehyde to 20,000 parts of juice.

Chloride of Lime.

This substance is recommended for disinfecting filter bags in breweries by H. Will ([5]), who advocates its use in the proportion of from 3 to 3½ kilos of chloride of lime per hectoliter of water. It is now extensively employed in the sugar industry for disinfecting bag filters, for which purpose it is generally employed in the strength of from one to two per cent.

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(1) Archief voor de Java Suikerindustrie, 1900. Bd. 8 S. 527.
(2) Handbook of Cane Sugar Manufacturers.
(3) Use of Formaldehyde in Sugar Mills, 1908.
(4) Bulletin de l'Association des Chimistes de Sucrerie et Dist., Vol. 25.
(5) Desinfektion und Desinfektionmittel im Brauereibetrieb.

Ammonium Fluoride.

Hydrofluoric acid and certain of its salts have a very strong germicidal action. The acid and its ammonium salt are used extensively in molasses distilleries for the prevention of the contamination of yeast with bacteria. Effront (1), who first introduced this use for fluorides, succeeded in acclimatizing yeast to as high a concentration of the acid as 36 grams per 100 liters. The ammonium salt which now is chiefly used for the purpose is employed in the proportion of 0.1 gram per liter. The use of ammonium fluoride for cleaning vats in distilleries and breweries is recommended by Jorgenson (2), who prescribes a 5% solution for this purpose. H. Winter (3) recommends the use of this substances in beet sugar factories as an excellent means of combatting the development of *Leuconostoc mesenteroides.* Saillard (4) recommends its use for preventing inversion of sucrose in the diffusion of batteries of beet sugar factories.

Sodium Fluoride.

Harloff (5) recommends the use of this substance in 1 per cent strength as a sugar factory germicide. He particularly advocates its use for cleaning the rolls of cane mills, as well as for the general germicidal needs to which milk of lime is at present put.

Milk of Lime.

Fresh milk of lime is recommended by Jorgensen (6) as a disinfectant for the walls and ceilings of breweries and distilleries. In the cane sugar industry it is extensively employed for cleaning the rolls of cane mills, and for all other germicidal purposes in connection with the cleaning of the factories. It has

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(1) Bulletin de l'Association des Chimistes de Sucrerie et Dist. Vol. 25.
(2) Microorganisms and Fermentation, p. 32.
(3) Archief voor de Java Suikerindustrie, 1893.
(4) Circulaire hebdom du Syndikat des fabr. de sucre, 1900. S. 610.
(5) Plantation White Sugar Manufacture, p. 23.
(6) Loc. cit.

been shown by Winter ([1]), however, that milk of lime is particularly ineffective in preventing the development of *Leuconostoc mesenteroides.* The explanation of its ineffectiveness in this connection is very probably due to the fact that this particular species of bacteria is favored by a neutral or slightly alkaline reaction, and hence the use of lime is apt to produce the conditions favorable for its growth.

Sodium and Calcium Bisulphite.

Both of these germicides have been recommended by Jorgensen ([2]) for use in breweries and distilleries. Saillard ([3]) successfully employed both of these substances for preventing the development of microörganisms in the diffusion batteries in beet factories. L. Lachaux ([4]) very strongly recommends the use of calcium bisulphite as a germicide for beet sugar factories.

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(1) Loc. cit.
(2) Loc. cit.
(3) Loc. cit.
(4) Bulletin de l'Association des Chimistes de Sucr. et Dist., 1893. Bd. 10 S. 501.

METHOD OF TESTING GERMICIDAL ACTION.

The various methods for testing the efficiency of germicides, including the original one used by Koch ([1]) on anthrax spores, and the various modifications that other investigators have made of this method, are not strictly applicable to the present investigation. These methods consist in exposing a test organism, either attached to silk threads, as in the original method used by Koch, or in the form of a fresh culture in nutrient solution, to the action of the germicide to be tested. At frequent intervals of time after the test organism is brought in contact with the germicide, transfers are made to a suitable culture medium, and the time required for the germicide to prevent the growth of the species is taken as the criterion of the germicidal value of the substance. In an investigation of the value of a germicide for sugar factory use, we are not exclusively concerned with its action upon any one species of microorganisms, but also, and chiefly, with its power to destroy the various species that usually occur in the products of the factory. It is necessary therefore to test the germicide upon these species collectively, in the product where they occur naturally, to determine the relative efficiency of germicides when used in sugar factories.

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(1) Mitt Kais. Ges. Amt., 1881. Bd. 1 S. 33.

The method selected for testing the action of the various germicides was as follows: Stock solutions of the substances were freshly prepared for every experiment. The strength of these solutions were such as to contain the desired amount of germicide in twenty ccs. For example, the stock formaldehyde solution which was to be used in 0.5% strength was made of 2.5% strength. The solution that was to be used in 5% strength was made up in 25% strength, so that in both cases the amount to be used was contained in 20 cc of the stock solution. In making the experiments 20 cc portions of the germicide solutions were introduced into 100 cc volumetric flasks. The flasks were then filled to the mark with the substance upon which the germicides were to be tested. A few minutes were allowed to elapse between the filling of the flasks, in order to allow sufficient time for the transfers of the substance from each flask immediately at the expiration of the desired period of exposure. The control flask received 20 ccs of sterile water and was filled to the mark, as in the other cases. The comparison with the control could then be made on an equal basis, since the amount of dilution was equalized in both cases. At the end of the period of exposure, transfers were made with sterile pipettes to flasks containing sterile water. From these, transfers were again made to sterile petri dishes, upon which sterile raw juice agar was poured, after which the plates were incubated for three days at a temperature of 35°C. During the time that the substance was exposed to the germicide an opportunity was given it to have been thoroughly mixed by frequently shaking the flasks. The dilution flasks were always thoroughly shaken before transfers were made from them. Six plates were made from every flask, and an average taken of the colony development upon them. Control plates were made in every case to determine whether plates and media were sterile.

The strengths of the stock solutions were based upon the composition of the substances and not upon their active principle. In the case of the formaldehyde solution, which was prepared from formalin, the strength is given in terms of formaldehyde, and not on the basis of the 40% commercial article. On the other hand, the strength of the chloride of lime solution is expressed in terms of the entire substance, and not on the available chlorine, which was approximately 35%.

Chapter II.

EXPERIMENTS WITH FRESH CANE JUICES.

The following table shows the results obtained with the use of the various germicides upon cane juice in the manner described in the previous chapter. The period of exposure in this experiment was twenty minutes, and the temperature at which the germicidal action took place was that of the room, which was 25°C. The results reported in this table are so entirely representative of a large number obtained from similar experiments that it was deemed unnecessary to report other results. The figures representing the efficiency of the germicides and their formaldehyde coefficient were derived in the following manner: The efficiency of germicidal action can best be expressed by dividing the number of microorganisms killed by the number in the original solution. In order to get the comparative efficiency of several germicides, the strength in which they are employed must be taken into account. Any arbitrary method of comparison, therefore, which takes into consideration the total germicidal action of a substance, and the amount required to perform it, will suffice for the needs of the present investigation. The following method was adopted: For every one per cent of microorganisms that survived an exposure to a one per cent solution of the germicide, ten was deducted from one hundred. Expressed as a formula, this becomes

$$E = 100 - \left(\begin{array}{c} 10 \\ \hline 1 \\ \hline S \end{array} \times \frac{L}{1} \right)$$

E = Efficiency.
S = Per cent strength of disinfectant.
L = Per cent microorganisms surviving the treatment.

It becomes apparent from this formula that E is increased or diminished according to the strength of the substance required for complete germicidal action. It is increased in proportion to which applications of less than one per cent strength are capable of destroying in the substance treated, all of the microorganisms in excess of one per cent. Conversely, it is decreased in proportion to which strengths in excess of one per cent are required to produce the same results. Under other circumstances the expression of germicidal efficiency on this basis would not be entirely correct. In pathology, for example, a survival of one per cent of the bacteria in a substance disinfected might give the disinfectant an efficiency of zero, since the surviving species might render the substance treated no less dangerous as a source of infection. In this investigation, however, we are more concerned with a rapid and extensive diminution of microorganisms, rather than with a complete sterilization process. For example, a one-half of one per cent solution of formaldehyde might decrease the microorganisms in a cane juice to one per cent of the original, in a fifteen minute exposure, while to destroy the remaining one per cent might require a several hours' exposure to a five per cent solution. It is obvious, therefore, that in such cases a complete sterilization would not justify the cost of the process.

Since formaldehyde is by far the most powerful of the germicides that we are testing, it is taken as the standard with which the others are to be compared. Its value is stated as 1, and the value of the others in terms of it, and their formaldehyde coefficient, is derived by the following formula:

$$\text{F. C.} = \frac{\text{L} \times \text{S F}}{\text{L} \times \text{Sx}}$$

F. C. = Formaldehyde coefficient.
L. = Per cent microorganisms surviving treatment.
S. F. = Per cent strength of formaldehyde.
Sx = Per cent strength of germicide to be tested.

TABLE I.

SHOWING RELATIVE EFFICIENCY OF VARIOUS GERMICIDES WHEN TESTED UPON FRESH CANE JUICES.

Germicide	% Strength	Dilution of Plate	(+000) No. of Micro. per c. c.	Efficiency	Formaldehyde Coefficient
Control	...	1 :1 mil.	13,000	....	....
Formaldehyde	0.5	1 :1000	30	98.85	1.
Sodium Bisulphite	5.0	1 :1000	152	42.0	.0198
Chloride of Lime	2.0	1 :1000	20	96.94	0.375
Milk of Lime	5.0	1 :1000	36	86.2	.0833
Sodium Fluoride	5.0	1 :1000	104	60.0	.0287
Ammonium Fluoride	5.0	1 :1000	25	90.4	0.119

Period of Exposure 20'.
Period of Incubation 72 hrs.

From the above table it will be noted that formaldehyde is, as was to be expected, far more efficient as a germicide than any of the others. Its germicidal action in .5 per cent strength is greater than the others in 5 per cent strength, with the exception of chloride of lime and ammonium fluoride. Milk of lime seems superior to both sodium bisulphite and sodium fluoride. The germicidal rank of the substances in the above experiment is as follows:

Formaldehyde.
Chloride of lime.
Ammonium fluoride.
Milk of lime.
Sodium fluoride.
Sodium bisulphite.

COMPARISON OF GERMICIDAL EFFICIENCY AT VARIOUS TEMPERATURES.

It is a well-known fact that the efficiency of germicides increases with elevations of the temperature at which they are allowed to act. That this accelerated action produced by elevated temperature varies with the germicidal substance is generally understood. In order to test the influence of elevated temperatures upon the various germicides, and to determine the range of temperature in which the acceleration of germicidal action was greatest, the following experiments were conducted. The methods employed in these experiments were the same as those previously described, except for the fact that the flasks in which the juice was treated were kept at a definite temperature during the period of exposure. After introducing the germicide into the flask, the juice was brought to the desired temperature by heating in a water bath and the flask filled to the mark with it, as before. After filling to the mark, the flasks were again placed in a water bath and kept at the desired temperature during the period of exposure.

Table II.

SHOWING GERMICIDAL ACTION OF FORMALDEHYDE UPON FRESH CANE JUICE AT VARIOUS TEMPERATURES.

Strength of Germicide	Temp.	Dilution of Plates	Average No. of Colonies	(+ 000) No. of Micro. per c. c.	Efficiency
Formaldehyde 0.5%	25° C	1:1000	51	51	97.5
Control	25° C	1:1 mil.	10	10,000	...
Formaldehyde 0.5%	35° C	1:1000	32	32	98.25
Control	35° C	1:1 mil.	9	9,000	...
Formaldehyde 0.5%	45° C	1:1000	18	18	98.88
Control	45° C	1:1 mil.	8	8,000	...
Formaldehyde 0.5%	55° C	1:1000	5	5	99.65
Control	55° C	1:100,000	70	7,000	...

Period of Exposure 15'.
Period of Incubation 72 hrs.

It will be noted from the table that the acceleration of the germicidal action of formaldehyde by elevated temperatures is very gradual. A rise of thirty degrees centigrade resulted in only 2.1% increase in germicidal efficiency, which is .7 of one per cent for each ten degrees' increase in temperature. In each case the comparison is made with a control exposed to the same temperature, in order that the detrimental action of the temperature might not be included in the germicidal action. The decrease in the number of microorganisms in the control juice resulting from the rise in temperature from 25°C. to 45°C. can scarcely be attributed solely to the effect of the temperature. It is probable that it is due to a large extent to the expansion of the solution, which would result in fewer numbers of microorganisms being contained in one cc than when measured at a lower temperature.

TABLE III.

SHOWING GERMICIDAL ACTION OF MILK OF LIME UPON FRESH CANE JUICE AT VARIOUS TEMPERATURES

Strength of Germicide	Temp.	Dilution of Plate	Average No. of Colonies	(+000) No. of Micro. per c. c.	Efficiency	Formaldehyde Coefficient
Milk of Lime 5%.......	25° C	1:1000	48	48	92.7	.351
Control................	25° C	1:1 mil.	33	33,000	....	....
Milk of Lime 5%.......	35° C	1:1000	25	25	96.5	.497
Control................	35° C	1:1 mil.	35	35,000	....	....
Milk of Lime 5%.......	45° C	1:1000	11	11	98.25	.635
Control................	45° C	1:1 mil.	31	31,000	....	....
Milk of Lime 5%.......	55° C	1:1000	8	8	98.35	.214
Control................	55° C	1:1 mil.	24	24,000	....	....

Period of Exposure 15'.
Period of Incubation 72 hrs.

From the above table it will be seen that the accelerative influence of elevated temperatures upon the germicidal action of milk of lime was greater than was true of formaldehyde. A rise of thirty degrees produced an increased efficiency of 5.6%, as compared to 2.1% for formaldehyde. The increase in germicidal efficiency is greatest from 25-35°C. Under the head of formaldehyde coefficient it will be seen that milk of lime more nearly approaches formaldehyde in germicidal value at 45°C., at which point its coefficient is 0.635. It is to be expected that the efficiency of every germicide increases with the temperature at which it acts. The fact that their maximum formaldehyde coefficient and their maximum efficiency are not always reached at the same temperature is due to the different rate at which the germicidal action of formaldehyde is accelerated at various temperatures. Thus, at 45°C., milk of lime has an efficiency of 98.25, as compared to 98.88 for formaldehyde, while at 55 the action of the former is only increased to 98.35, while the latter attains an efficiency of 99.65.

TABLE IV.

SHOWING GERMICIDAL ACTION OF SODIUM BISULPHITE UPON FRESH JUICE AT VARIOUS TEMPERATURES.

Strength of Germicide	Temp.	Dilution of Plate	Average No. of Colonies	(+000) No. of Micro. per c. c.	Efficiency	Formaldehyde Coefficient
Sodium Bisulphite 5%..	25° C	1:1000	477	477	....	.0106
Control..................	25° C	1:1 mil.	10	10,000	....	....
Sodium Bisulphite 5%..	35° C	1:1000	447	447	....	.0074
Control..................	35° C	1:1 mil.	10	10,000	....	....
Sodium Bisulphite 5%..	45° C	1:1000	173	173	13.50	.0130
Control..................	45° C	1:1 mil.	10	10,000	....	....
Sodium Bisulphite 5%..	55° C	1:1000	105	105	42.	.00615
Control..................	55° C	1:1 mil.	9	9,000	....	....

Period of Exposure 15'.
Period of Incubation 48 hrs.

From the above table it will be seen that sodium bisulphite has a comparatively weak germicidal action even at elevated temperatures. At temperatures below 45°C. its efficiency, as derived from the formula, is negative. A very striking fact in connection with the effect of elevated temperatures upon the germicidal action is shown in the increase of efficiency produced by a rise in temperature from 25 to 35°C. From a negative efficiency at 25, it reached 13.5 at 45, and is more than tripled by an increase of ten degrees from 45 to 55. We might regard 35°C. as the critical temperature for the germicidal efficiency of sodium bisulphite. No doubt this difference is to be attributed to the greater liberation of SO_2 at this temperature.

TABLE V.

SHOWING GERMICIDAL ACTION OF SODIUM FLUORIDE UPON FRESH JUICE AT VARIOUS TEMPERATURES.

Strength of Germicide	Temp.	Dilution of Plate	Average No. of Colonies	(+000) No. of Micro. per c. c.	Efficiency	Formaldehyde Coefficient
Sodium Fluoride 5%....	25° C	1:1000	333	333	....	.0228
Control................	25° C	1:1 mil.	15	15,000	....	....
Control................	25° C	1:40,000	370	14,800	....	....
Sodium Fluoride 5%....	35° C	1:1000	393	393	....	.0126
Control................	35° C	1:1 mil.	14	14,000	....	....
Sodium Fluoride 5%....	45° C	1:1000	343	343	....	.00723
Control................	45° C	1:1 mil.	11	11,000	....	....
Sodium Fluoride 5%....	55° C	1:1000	20	20	90.95	.0394
Control................	55° C	1:1 mil.	10	11,000	....	....

From the above table it will be noted that Sodium Fluoride has a negative germicidal efficiency as measured by the formula $E = 100 - \left(\frac{10}{\frac{1}{S}} \times \frac{L}{1}\right)$ at temperature under 55° C. The acceleration of efficiency between 45° C and 55° C is very striking.

Period of Exposure 15′.
Period of Incubation 72 hrs.

TABLE VI.

SHOWING GERMICIDAL ACTION OF CHLORIDE OF LIME UPON FRESH JUICE AT VARIOUS TEMPERATURES.

Strength of Germicide	Temp.	Dilution of Plate	Average No. of Colonies	(+000) No. of Micro. per c. c.	Efficiency	Formaldehyde Coefficient
2% Chloride of Lime....	25° C	1:1000	18	18	91.0	.283
Control................	25° C	1:1 mil.	4	4,000	....	....
2% Chloride of Lime....	35° C	1:1000	9	9	95.56	.398
Control................	35° C	1:1 mil.	4	4,000	....	....
2% Chloride of Lime....	45° C	1:1000	7	7	96.5	.321
Control................	45° C	1:1 mil.	4	4,000	....	....
2% Chloride of Lime....	55° C	1:1000	4	4	98.0	.178
Control................	55° C	1:1 mil.	4	4,000	....	....

It will be seen from the above table that Chloride of Lime is highly efficient as a germicide even at low temperatures. Its maximum formaldehyde coefficient is attained at 35° C. Its maximum efficiency was 98.0, which was reached at 55° C.

Period of Exposure 15′.
Period of Incubation 72 hrs.

TABLE VII.

SHOWING GERMICIDAL ACTION OF AMMONIUM FLUORIDE AT VARIOUS TEMPERATURES.

Strength of Germicide	Temp.	Dilution of Plate	Average No. of Colonies	(+000) No. of Micro. per c. c.	Efficiency	Formaldehyde Coefficient
Ammonium Fluoride 5%	25° C	1:1000	19	19	84.2	.161
Control	25° C	1:1 mil.	6	6,000	....	....
Ammonium Fluoride 5%	35° C	1:1000	13	13	90.75	.191
Control	35° C	1:1 mil.	7	7,000	....	....
Ammonium Fluoride 5%	45° C	1:1000	7	7	95.0	.225
Control	45° C	1:1 mil.	7	7,000	....	....
Ammonium Fluoride 5%	55° C	1:1000	5	5	95.0	.071
Control	55° C	1:1 mil.	5	5,000	....	....

It will be noticed from the above table that Ammonium Fluoride differs from the other germicides in the fact that its maximum efficiency is attained at 45° C. Its maximum formaldehyde coefficient is attained at this temperature also.

Period of Exposure 15′.
Period of Incubation 72 hrs.

TABLE VIII.

SUMMARIZING INFLUENCE OF TEMPERATURE AND LENGTH OF EXPOSURE UPON THE EFFICIENCY AND FORMALDEHYDE COEFFICIENTS OF VARIOUS GERMICIDES.

GERMICIDE	Influence of Temperature upon Efficiency				Influence of Length of Exposure	
	Max. Eff.	Temp. for Max. Eff.	Max. Form. Coeff.	Temp. for Max. Form. Coeff.	Max. Form. Coeff.	Time for Max. Form. Coeff.
Formaldehyde, 0.5%	99.65	55	...	...	...	...
Chloride of Lime, 2%	98.0	55	.398	35	.375	20′
Ammonium Fluoride, 5%	95.0	45	.225	45	.225	30′
Milk of Lime, 5%	98.35	55	.633	45	.615	45′
Sodium Fluoride, 5%	90.95	55	.0394	55	.0287	20′
Sodium Bisulphite, 5%	42.0	55	.0130	45	.0198	20′

Referring to the above table, it will be noted that ammonium fluoride alone of all the germicides tested reaches its maximum efficiency at a temperature lower than the maximum temperatures employed in the experiments. While with this exception the maximum efficiency of all the germicides coincides with the maximum temperature employed, yet their formaldehyde coefficient reaches its maximum in almost every case at the lower temperatures. As has been previously pointed out, this is due to the fact that the rate of acceleration of the germicidal action of formaldehyde by elevations in temperatures does not coincide with that produced on the other germicides. As a result of this different rate of acceleration, we have a maximum efficiency almost invariably at the maximum temperature, but a maximum formaldehyde coefficient at the lower temperature. It will be noted in the above table that the rank taken by the various germicides, as based upon their relative maximum efficiency, is different from that shown in Table I. This is due to the fact that the comparison in that table was made at room temperature, while here their relative efficiency is compared at 45°C. and 55°C.

The data shown in the column headed ''Influence of Length of Exposure'' was obtained from a series of special experiments. It was thought probable that the comparative efficiency of the weaker germicides and formaldehyde might increase with the length of exposure. In most of the cases, however, the maximum formaldehyde coefficient was attained in the period of shortest exposure. Milk of lime is apparently an exception to this rule, and is more nearly comparable with formaldehyde in its efficiency in a 45′ exposure.

COMPARISON OF EFFICIENCY OF GERMICIDES UPON FERMENTED JUICES.

We have previously referred to the fact that the efficiency of a germicide varies not only with the various species of micro-organisms upon which it may be tested, but also with the composition of the substance with which it must be brought in contact to perform its work. Our previous experiments were conducted upon fresh juices only. That the efficiency of the germicides might be quite different when tested upon fermented juices might easily be assumed upon the grounds stated above. Not only is the composition of the substance different in the two cases, but the species of microorganisms predominating may also differ, which would further influence the efficiency of the germicides. It was considered necessary, therefore, to test the germicides upon fermented juices, in order to ascertain whether their efficiency was the same under these conditions as when used on fresh juices. For these experiments two types of fermented juices were selected, which represented two stages of fermentation. The partly fermented juice was one which had been allowed to ferment for twelve hours in the incubator, while the badly fermented juice was one which had been kept on hand for some time in the laboratory and was completely fermented. The method of treating them with the germicidal agents was the same as that previously described.

TABLE IX.

SHOWING COMPARATIVE EFFICIENCY OF FORMALDEHYDE UPON FERMENTED JUICES.

	Slightly Fermented Juice		Badly Fermented Juice	
Plate	Formaldehyde	Control	Formaldehyde	Control
	(+ 000) No. of Micro. per c. c.	(+ 000) No. of Micro. per c. c.	(+ 000) No. of Micro. per c. c.	(+ 000) No. of Micro. per c. c.
1	120	7,000	1,220	140,000
2	80	8,000	1,200	140,000
3	80	12,000	1,040	120,000
4	80	9,000	1,220	112,000
5	100	7,000	1,100	140,000
6	80	7,000	1,160	160,000
Ave.	90,000	8,000	1,157,000	135,000,000

Efficiency of Slightly Fermented Juice.............	94.40
Efficiency on Badly Fermented Juice..............	95.75
Dilution of Control.............................	1 :1 million
Dilution of Formaldehyde Treated Samples........	1 :1000

It will be noted from the above table that the germicidal efficiency of formaldehyde is greater on badly fermented juices than upon juice that is only partly fermented. This is perhaps due to the fact that in the former there is a greater preponderance of less resistant species of microorganisms, such as yeast and non-sporulating bacteria. If we compare the germicidal efficiency of formaldehyde on fermented juice with that derived in our experiments on fresh juice, we find, however, that its germicidal action does seem to be impaired by the products formed during fermentation. Referring to Table I, we find that formaldehyde has a germicidal efficiency on fresh juice of 98.85, while on badly fermented juice its efficiency is only 95.71. This decrease in germicidal efficiency may be due to one or to a combination of several causes. It may be due to certain combinations that the formaldehyde forms with the products of fermentation. The mere excessive numbers of microorganisms in the badly fermented juice might of itself tend to lower the germicidal efficiency of a substance. Since the action of most germicides depends upon a definite reaction taking place between them and the microorganisms, which they kill, it is evident that the greater the number of microorganisms the greater will be the quantity of germicide required. There are, of course, certain exceptions to this rule, for some germicides act as catalytic poison, which kill by mere contact and do not depend upon forming chemical combinations for their germicidal action. Prominent among this class of germicides are chloroform, ether, carbon bisulphide, chloral, etc. Formaldehyde, however, like all aldehydes, owes its germicidal action to the ease with which it reacts with the cell substance of microorganisms. It acts readily upon albumen, forming insoluble compounds. According to Loew (1), the toxicity of all aldehydes is directly proportionate to their instability, the most unstable ones having the greatest toxicity.

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(1) Ein Naturliches System der Giftwirkungen.

TABLE X.

SHOWING COMPARATIVE EFFICIENCY OF CHLORIDES OF LIME UPON FERMENTED JUICES.

Plate	Slightly Fermented Juice		Badly Fermented Juice	
	Chloride of Lime 2%	Control	Chloride of Lime 2%	Control
	(+ 000) No. of Micro. per c. c.	(+ 000) No. of Micro. per c. c.	(+ 000) No. of Micro. per c. c.	(+ 000) No. of Micro. per c. c.
1	20	7,000	27	140,000
2	18	8,000	20	140,000
3	18	12,000	20	120,000
4	17	9,000	30	112,000
5	17	7,000	23	140,000
6	18	7,000	29	160,000
Ave.	18,000	8,000,000	25,000	135,000,000

Efficiency Slightly Fermented Juice	95.5
Formaldehyde Coefficient	1.24
Efficiency Badly Fermented Juice	99.63
Formaldehyde Coefficient	11.5
Dilution of Control	1 :1 million
Dilution of Treated Sample	1 :1000

The increased germicidal efficiency of chloride of lime when acting upon sour juices is very striking. This significant increase in germicidal efficiency under these conditions is most probably due to the greater liberation of chlorine from the chloride of lime by the action of the acids developed in the course of the fermentation of the juice. In this case, however, just as in the case of formaldehyde, the greater efficiency upon fermented juice is, no doubt, in part due to the preponderance of species of low resistance in the badly fermented juice. In both of the fermented juices chloride of lime is superior to formaldehyde. Its formaldehyde coefficient on the badly fermented juice is 11.5, which means that under these conditions chloride of lime is much superior to formaldehyde as a germicide.

TABLE XI.

SHOWING COMPARATIVE EFFICIENCY OF SODIUM BISULPHITE UPON FERMENTED JUICES.

Plate	Slightly Fermented Juice		Badly Fermented Juice	
	Sodium Bisulphite 5%	Control	Sodium Bisulphite 5%	Control
	(+ 000) No. of Micro. per c. c.	(+ 000) No. of Micro. per c. c.	(+ 000) No. of Micro. per c. c.	(+ 000) No. of Micro. per c. c.
1	960	7,000	760	140,000
2	800	8,000	740	140,000
3	620	12,000	660	120,000
4	760	9,000	600	112,000
5	720	7,000	600	140,000
6	900	7,000	800	160,000
Ave.	793,000	8,000,000	694,000	135,000,000

Efficiency Slightly Fermented Juice	....
Formaldehyde Coefficient	0.0113
Efficiency Badly Fermented Juice	74.3
Formaldehyde Coefficient	0.166
Dilution of Control	1:1 million
Dilution of Treated Sample	1:1000

It will be noted from the above table that the germicidal efficiency of sodium bisulphite is greater with badly fermented juices than with the slightly fermented. Compared with its germicidal efficiency on fresh juice, as shown in Table I, it is almost twice as efficient. The greater efficiency of sodium bisulphite on fermented juices is to be attributed to a greater liberation of sulphur dioxide in an acid solution. The same explanation doubtless applies in this case as in the case of chloride of lime.

TABLE XII.

SHOWING COMPARATIVE EFFICIENCY OF AMMONIUM PLUORIDE UPON FERMENTED JUICES.

Plate	Slightly Fermented Juice		Badly Fermented Juice	
	Ammonium Fluoride 5%	Control	Ammonium Fluoride 5%	Control
	(+ 000) No. of Micro. per c. c.	(+ 000) No. of Micro. per c. c.	(+ 000) No. of Micro. per c. c.	(+ 000) No. of Micro. per c. c.
1	10	14,000	220	60,000
2	10	13,000	200	77,000
3	10	10,000	140	78,000
4	7	12,000	180	71,000
5	7	15,000	160	66,000
6	10	12,000	140	79,000
Ave.	9,000	13,000,000	173,000	71,000,000

Efficiency Slightly Fermented Juice................	96.54
Formaldehyde Coefficient..........................	1.61
Efficiency Badly Fermented Juice..................	87.85
Formaldehyde Coefficient..........................	0.353
Dilution of Control................................	1 :1 million
Dilution of Treated Sample.......................	1 :1000

It is apparent from the above table that the germicidal efficiency of ammonium fluoride is materially impaired by contact with badly fermented juice. Its germicidal efficiency on slightly fermented juice, however, is greater than on fresh juice, as shown in Table I.

Table XIII.

SHOWING COMPARATIVE EFFICIENCY OF SODIUM FLUORIDE UPON FERMENTED JUICES.

Plate	Slightly Fermented Juice		Badly Fermented Juice	
	Sodium Fluoride 5%	Control	Sodium Fluoride 5%	Control
	(+ 000) No. of Micro. per c. c.	(+ 000) No. of Micro. per c. c.	(+ 000) No. of Micro. per c. c.	(+ 000) No. of Micro. per c. c.
1	620	14,000	3,300	60,000
2	700	13,000	2,000	77,000
3	700	10,000	5,000	78,000
4	620	12,000	3,500	71,000
5	800	15,000	3,500	66,000
6	600	12,000	2,500	79,000
Ave.	673,000	13,000,000	3,300,000	71,000,000

Efficiency Slightly Fermented Juice	....
Formaldehyde Coefficient	.0214
Efficiency Badly Fermented Juice	....
Formaldehyde Coefficient	.0184
Dilution of Control	1:1 million
Dilution of Treated Sample	1:1000

From the above table it will be noted that sodium fluoride, like ammonium fluoride, has a lower germicidal efficiency on fermented juice than on fresh juice.

Table XIV.

SHOWING COMPARATIVE EFFICIENCY OF BISULPHIDE OF LIME UPON FERMENTED JUICES.

Plate	Slightly Fermented Juice		Badly Fermented Juice	
	Bisulphite of Lime 5%	Control	Bisulphite of Lime 5%	Control
	(+ 000) No. of Micro. per c. c.	(+ 000) No. of Micro. per c. c.	(+ 000) No. of Micro. per c. c.	(+ 000) No. of Micro. per c. c.
1	660	14,000	780	60,000
2	680	13,000	480	77,000
3	680	10,000	660	78,000
4	520	12,000	640	71,000
5	640	15,000	620	66,000
6	...	12,000	...	79,000
Ave.	636,000	13,000,000	636,000	71,000,000

Efficiency Slightly Fermented Juice................	....
Formaldehyde Coefficient..........................	.0224
Efficiency Badly Fermented Juice..................	55.25
Formaldehyde Coefficient..........................	.0956
Dilution of Control..............................	1 :1 million
Dilution of Treated Sample.......................	1 :1000

From the above table it will be seen that calcium bisulphite, like sodium bisulphite, has a higher germicidal efficiency on badly fermented juice than on slightly fermented juice. The explanation of this is the same as was stated in the former case, viz., that there is a greater liberation of sulphur dioxide in the more acid solution. Comparing the germicidal efficiency of the two bisulphites, we find their relative efficiency to be almost in direct proportion to their SO_2 content.

TABLE XV.

SHOWING COMPARATIVE EFFICIENCY OF MILK OF LIME UPON FERMENTED JUICES.

Plate	Slightly Fermented Juice		Badly Fermented Juice	
	Milk of Lime 5%	Control	Milk of Lime 5%	Control
	(+ 000) No. of Micro. per c. c.	(+ 000) No. of Micro. per c. c.	(+ 000) No. of Micro. per c. c.	(+ 000) No. of Micro. per c. c.
1	58	14,000	66	60,000
2	...	13,000	75	77,000
3	50	10,000	74	78,000
4	50	12,000	60	71,000
5	60	15,000	54	66,000
6	50	12,000	81	79,000
Ave.	54,000	13,000,000	68,000	71,000,000

Efficiency Slightly Fermented Juice	79.25
Formaldehyde Coefficient	0.270
Efficiency Badly Fermented Juice	95.25
Formaldehyde Coefficient	0.896
Dilution of Control	1:1 million
Dilution of Treated Sample	1:1000

Milk of lime also seems to have a higher germicidal efficiency on fermented juice than on fresh juice. Comparing its efficiency on badly fermented juice, as shown in the above table, with that given in Table I, we have an efficiency of 95.21 in the former, as against 86.2 in the latter.

THE COMPARATIVE EFFICIENCY OF GERMICIDES UNDER CONDITIONS WHICH INVOLVE THEIR PENETRATIVE POWERS.

Some germicides which are highly efficient under conditions which do not require any power of penetration on their part in order for them to come in immediate contact with the microorganisms to be destroyed are relatively impotent under conditions which involve such properties. On account of this fact, we often find that the most powerful germicides can sometimes be substituted to advantage in such cases by ones that are weaker under ordinary conditions. As a result of this variation in penetrative power on the part of germicides, we find that some of the less powerful ones are strongly recommended for cleaning vats and tanks in distilleries. In such cases it is highly important for the germicide to have the power of penetrating the minute crevices in the surfaces of the vats, in order for their germicidal powers to reach the microorganisms contained therein. It is obvious that the conditions under which our previous experiments upon the efficiency of germicides on juices were conducted are not strictly comparable to sugar house conditions, if we consider the importance of the penetrating power of these substances. In the previous experiments the juice and germicides were mixed in volumetric flasks. Under these conditions the inner surfaces of the container were so smooth as to offer no difficulties for a thorough contact to be established between germicide and juice at the sides of the container. Such ideal conditions do not obtain in the apparatus of sugar factories, where the unevenness of the inner surfaces furnishes favorable points for the development of microorganisms where they are not easily reached by germicides. In order to test the efficiency of the germicides under conditions that more closely approximate those in the sugar factory, the following experiments were conducted. Instead of treating the juice in flasks, two-gallon wooden buckets were used for the purpose. One liter of fermented juice was poured into each bucket and allowed to remain for thirty minutes before applying the germicide. Two hundred ccs of the germicide solution was then poured into the bucket, and the contents then thoroughly mixed by stirring. To the bucket containing the control juice two hundred ccs of sterile water was added and mixed, as in the other cases. After the germicides had remained in contact with the juice for fifteen minutes, the contents of the buckets were poured off and the buckets were inverted and allowed to drain for two minutes. They were then turned up in their normal position and 500 ccs of sterile water added to each bucket and thoroughly mixed with the contents that had adhered to the sides. After the insides of each bucket had been thoroughly washed down, transfers were made to flasks of sterile water and plates made from them in the usual way. Although there are relatively large factors of error in an experiment of this kind, it was thought that the results would show, in a general way, the facts that were intended to be brought out. One of the largest factors of error would naturally be the variations in the actual amount of juice left in each bucket and the different degrees of dilution that the addition of the 500 ccs of water would represent in each case. The opportunity for the buckets to drain were identical in each case, so far as the time allowed for this purpose and the position of the buckets were concerned. The same juice was used for each bucket, so the rate at which it would drain from the sides should have been apparently the same in each case, except that the viscosity of the juice might have varied with the addition of the various germicides.

Table XVI.

SHOWING COMPARATIVE EFFICIENCY OF GERMICIDES UPON JUICES IN WOODEN PAILS.

Series Disinfectant	% Strength	Average No. of Colonies	(+000) No. of Micro. per c. c.	Efficiency	Formaldehyde Coefficient
Control A	...	18	3,600+	....	....
Control B	...	14	2,800+	....	....
Formaldehyde	0.5%	73	73+	88.60	1.
Sodium Bisulphite	5.0%	39	39+	39.50	0.188
Milk of Lime	5.0%	13	13+	79.70	0.561
Chloride of Lime	2.0%	8	8+	95.00	2.08
Ammonium Fluoride	5.0%	4	4+	93.75	1.82
Sodium Fluoride	5.0%	198	198+	....	0.362

Time allowed to act	15′
Incubation Period	72 hrs.
Dilution of Control	1:200,000
Dilution of Treated Sample	1:100,000

The above table shows that the germicidal efficiency of the various substances is quite different under the conditions of this experiment. Under these conditions it appears that both ammonium fluoride and chloride of lime have a higher germicidal efficiency than formaldehyde. Formaldehyde shows a comparatively low germicidal efficiency in this experiment, which is no doubt to be attributed to its lack of penetrative power, and also, but to a lesser extent, to its lower efficiency on fermented juice. Chloride of lime has a formaldehyde efficiency of 2 in this experiment, which indicates that where the penetrative power of the germicide is involved this substance is superior to formaldehyde. It is perhaps on account of the penetrative power of chloride of lime that it is so strongly recommended for cleaning filter bags in sugar factories.

Table XVII.

SHOWING COMPARATIVE EFFICIENCY OF GERMICIDES UPON JUICES IN WOODEN PAILS.

Disinfectant Used	% Strength	Average No. of Colonies	(+000) No. of Micro. per c. c.	Efficiency	Formaldehyde Coefficient
Formaldehyde	0.5%	196	196+	95.5	1.
Sodium Bisulphite...........	5.0%	212	212+	51.4	0.0925
Chloride of Lime............	2.0%	19	19+	98.26	2.58
Milk of Lime................	5.0%	17	17+	96.10	1.15
Sodium Fluoride.............	5.0%	110	110+	79.4	0.218
Ammonium Fluoride........	5.0%	180	180+	58.75	0.109
Control	...	212	21,200+	....	....
Control......................	...	112	22,400+	....	....

Time allowed to act..............	15′
Incubation Period.................	72 hrs.
Dilution of Control...............	1 :1,000,000 and 1 :200,000
Dilution of Treated Sample.......	1 :1000

The juice used in the above experiment was quite different from that employed in the previous case. This juice was not so completely fermented, but was very viscous as a result of a vigorous dextran fermentation that had taken place in it. As a result of this greater viscosity of the juice it would seem that the efficiency of the germicides in this case would be even more dependent upon their penetrative power than in the previous experiment. In this case the juice would adhere to the sides more tenaciously, and it would be more difficult for the germicides to come in through contact with it.

The results of this experiment are somewhat different from those obtained in the preceding one. In this case formaldehyde is surpassed in germicidal efficiency by chloride of lime and milk of lime. The formaldehyde coefficient of the former is very nearly the same in both experiments. The germicidal efficiency of formaldehyde seems to be higher in this experiment than in the preceding one. From these experiments it seems probable that formaldehyde can well be dispensed with in favor of chloride of lime as a germicide for use in certain special cases in the cleaning of sugar factories. In order, however, to ascertain the relative expense connected with the use of the two substances, their relative germicidal efficiency must be taken in connection with the prices at which they can be procured. This comparison will be taken up in a subsequent chapter.

THE STIMULATIVE ACTION OF SUBLETHAL APPLICATIONS OF VARIOUS GERMICIDES UPON THE MICROORGANISMS OF CANE JUICE.

The theory that cell development may be stimulated by the application of toxic substances in quantities insufficient to produce death was first proposed as a biological law by Arndt (1) in his work, "Biologische Studien." The application of this law to microorganisms has been conclusively established by the investigations of Hüne (2), Koch (3), Fred (4), Schulze (5), Raulin (6), Nageli (7), Kruger (8) and others. In a recent investigation by Meade (9) the application of this law to the microorganisms in cane sugar products has been very clearly demonstrated. As Meade relied solely upon chemical analyses to determine the relative stimulative power of various germicides, it seemed that the duplication of a part of his work by bacteriological instead of chemical methods might be of some scientific value. Since only a few of the germicides used in this investigation were tested by the author above referred to, the following experiments are not an entire duplication of the previous work.

The investigation of the stimulative action of the various germicides upon the microorganisms in cane juice is warranted in the scope of the present work solely upon the grounds that it possesses scientific interest. It is not calculated to yield results that are susceptible of any practical application in connection with the use of germicides in sugar factories. The demonstration by Meade of the stimulative action of sugar factory germicides was quite accidental, since he was primarily investigating the relative value of different substances as preservatives for laboratory samples when he observed that his treated samples deteriorated more rapidly than the controls.

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(1) Biologische Studien I.
(2) Cent. fur Bakt. Abt. I. Bd. 48, 1909.
(3) Cent. fur Bakt. Abt. II. Bd. 31.
(4) Cent. fur Bkt. Abt. II. Bd. 31.
(5) Pflugers Archives. Bd. 42, 1888.
(6) These, Paris, 1810.
(7) Schweizer Gesellsch, f. d. gesamt. Naturwissenschaft. Bd. 33, 1893.
(8) Cent. fur Bakt. Abt. II. Bd. I, p. 10.
(9) Action of Disinfectants on Sugar Solutions. La. Planter, 1912, p 7349.

The method of conducting the experiments was as follows: The juice and germicides were mixed, as in the previous experiments. After a period of exposure of fifteen minutes, the juice was transferred to dilution flasks, plated out and incubated for one week. A comparison of the untreated juice and those portions of it which had been treated with the germicides showed the initial depressing action of the treatment. The flasks containing the treated juices were then incubated for twenty-four hours at a temperature of 35°C., at the expiration of which time they were again plated out and compared with an untreated control.

TABLE XVIII.

PRELIMINARY EXPERIMENT UPON THE STIMULATIVE ACTION OF SMALL QUANTITIES OF GERMICIDE UPON THE MICROORGANISMS OF CANE JUICE.

Germicide	15 Minutes After Addition of Germicide			After 24 Hours	
	Strength	Average No. of Colonies	(+000) No. of Micro. per c. c.	Average No. of Colonies	(+000) No. of Micro. per c. c.
Control	....	41	8,200	167	167,000
Formaldehyde..................	1:50,000	24	4,800	60	60,000
Milk of Lime..............	1:200	11	2,200	5	1,000
Chloride of Lime...........	1:5,000	13	2,600	84	16,800
Ammonium Floride........	1:5,000	25	5,000	97	19,400
Sodium Bisulphite.........	1:200	45	9,000	10	2,000
Calcium Bisulphite.........	1:200	29	5,800	8	1,600
Sodium Fluoride...........	1:200	25	5,000	20	4,000

Dilution of Control.............	1:200,000 and 1:1 million
Dilution of Treated Sample.....	1:200,000 and 1:200,000

The above table shows the results of a preliminary experiment, the purpose of which was to ascertain the strengths in which the various germicides produce a stimulative action. It will be observed from this table that the strengths of the germicides were too great, and that the number of microorganisms in the control flask exceeded those in the treated samples.

Meade ([1]) found formaldehyde to be stimulative upon the microorganisms in raw sugar washings when used in the strength of 1:50,000. However, this dilution was made on the basis of formalin, which contains 40% formaldehyde, thus representing a formaldehyde dilution of 1:125,000.

TABLE XIX.

SHOWING THE STIMULATIVE ACTION OF SMALL QUANTITIES OF GERMICIDES UPON THE MICROORGANISMS OF CANE JUICE.

Germicide	15 Minutes After Addition of Germicide			After 24 Hours	
	Strength	Average No. of Colonies	(+000) No. of Micro. per c. c.	Average No. of Colonies	(+000) No. of Micro. per c. c.
Control	....	21	4,200	47	47,000
Formaldehyde.........	1:100,000	22	4,400	81	81,000
Milk of Lime..............	1:1,000	24	4,800	27	27,000
Chloride of Lime..........	1:20,000	18	3,600	135	135,000
Ammonium Fluoride.......	1:20,000	19	3,800	85	85,000
Sodium Bisulphide.........	1:1,000	23	4,600	46	46,000
Calcium Bisulphite.........	1:1,000	21	4,200	73	73,000
Sodium Fluoride...........	1:1,000	30	6,000	14	14,000

Dilution of Control............. 1:200,000 and 1:1 million
Dilution of Treated Sample..... 1:200,000 and 1:1 million

In the above table it will be noted that formaldehyde 1:100,000, chloride of lime 1:20,000 and ammonium fluoride 1:20,000 exerted a marked stimulative action upon the microorganisms of cane juice. The degree of stimulation by chloride of lime is especially marked. The results in this experiment approximately agree with those of Meade, which showed the maximum stimulative strength of formaldehyde to be 1:125,000, chloride of lime from 1:10,000 to 1:20,000, and the same for ammonium fluoride. Of the remaining germicides only bisulphite of lime showed any stimulative action. Sodium bisulphite did not seem to decrease the number of organisms in the juice to any appreciable extent. It is interesting to note that only the stronger germicides seemed capable of exercising a stimulative action upon the microorganisms in juices.

Chapter III.

EXPERIMENTS UPON SYRUPS AND SUGARS.

We have already observed that the efficiency of germicides is influenced by the composition of the substance upon which they are used. Since the composition of syrups and sugars present very different conditions for the action of germicides from those obtained in our previous experiments, it is necessary to compare the efficiency of the germicides under these conditions. These substances differ from juices not only in their composition but also in the microorganisms that they contain. In cane juices the predominant species are usually of low resistance to heat and to germicidal agents. The predominant species in sugars and syrups are, on the contrary, spore forming bacteria that are highly resistant to heat and to germicides. In order to test the efficiency of the various germicides upon these products the following experiments were conducted: A syrup which was badly fermented was selected for the experiments. It had an original density of 74 Brix, which had decreased to 65 at the time of the experiment. The method of treating it was the same as that described for the treatment of juices.

TABLE XX.

SHOWING RELATIVE EFFICIENCY OF VARIOUS GERMICIDES WHEN TESTED UPON CANE SYRUP (FERMENTING SYRUP AT 15° C.).

Substance Employed	% Strength	Average No. of Colonies	(+000) No. of Micro. per c. c.	Efficiency	Formaldehyde Coefficient
Control	...	702	702,000	....	....
Formaldehyde	0.5%	13	13,000	90.75	1.
Sodium Bisulphide	10.0%	72	72,000	....	0.009
Chloride of Lime	2.0%	11	11,000	68.8	0.296
Milk of Lime	5.0%	17	17,000	....	0.0764
Sodium Fluoride	5.0%	131	131,000	....	0.0099
Ammonium Fluoride	5.0%	12	12,000	14.5	0.108
Sulphite of Lime	5.0%	15	15,000	....	0.086

Period of Exposure.............................. 15′
Period of Incubation............................ 72 hrs.
Dilution of Plates.............................. 1:1000

From the above table it will be seen that all of the germicides have a comparatively low efficiency on syrups. Only three of the germicides showed a positive efficiency, and the formaldehyde coefficients are low in every case. The efficiency of formaldehyde, although far greater than any of the others, is very low in comparison with the results obtained on juice.

For the experiments on sugar a badly deteriorated sugar was selected and a fifty per cent solution made with water. The method of treating with the various germicides was the same as in the previous experiments.

TABLE XXI.

SHOWING RELATIVE EFFICIENCY OF VARIOUS GERMICIDES WHEN TESTED UPON DETERIORATED SUGAR.

Germicides	% Strength	Average No. of Colonies	(+000) No. of Micro. per c. c.	Efficiency	Formaldehyde Coefficient
Control	...	48	48,000	....	....
Formaldehyde.......	0.5%	8	8,000	....	1.
Chloride of Lime...........	2%	9	9,000	....	0.221
Ammonium Fluoride........	5%	5	5,000	....	0.159
Calcium Bisulphite..........	5%	15	15,000	....	0.532
Sodium Bisulphite...........	10%	25	25,000	....	0.0159
Sodium Fluoride............	5%	14	14,000	....	0.0570
Milk of Lime...............	5%	10	10,000	....	0.0798

Period of Exposure..............................	15′
Period of Incubation..............................	72 hrs.
Dilution of Plates..................................	1:1000

In this experiment only formaldehyde showed a positive efficiency, and it was exceedingly low. The formaldehyde coefficients were without exception very low. An interesting point that developed during the course of these investigations was the variation in the germicide efficiency of formaldehyde on different solutions. In the two tables given above, the germicidal efficiency of formaldehyde, although comparatively low, is much higher than the results obtained from experiments on other samples of syrups and sugars. It appears probable that the low efficiency in the other cases was due to the fact that certain combinations were formed between the formaldehyde and certain bodies contained in the solution. That such combination may take place is suggested by Norris ([1]), who attributes the influence of formaldehyde upon the polarization of sugar solutions to a reaction taking place between it and the sugar. To quote the author on the subject, we have the following: "The most probable explanation of the reaction between the formaldehyde and the sugar seems to the writer to be the formation of an unstable compound of the two, which has a higher polarization than the sugar itself. The formation of such combinations is also suggested in the work of Yoder and Taggart." ([2])

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(1) Loc. cit.

(2) Occurrence of Formaldehyde in Sugar House Products. Int. Sugar Jour., 1910.

Chapter IV.

THE DETERMINATION OF THE PHENOL CO-EFFICIENT OF THE VARIOUS GERMICIDES.

The usual method for the standardization of disinfectants in public health work is by determining their phenol coefficient. Phenol is selected as a standard for these comparisons owing to the fact that its action is less affected by the presence of organic matter. In order to compare the efficiency of the germicides employed in the preceding experiments, on the same basis as that used in medical investigations, an attempt was made to establish the phenol coefficient for each of the germicides.

There are several methods now in use for the determination of the phenol coefficient of germicides. The most important of these may be briefly described as follows: The Rideal Walker(1) method, which was one of the first employed for this purpose, consists in exposing a 24-hour culture of the test organism to the action of various dilutions of phenol and the germicide to be tested. The phenol coefficient is then derived by dividing the figure indicating the degree of dilution of the disinfectant that kills the organism in a given time by that expressing the degree of dilution of the phenol that kills the same organism in the same time under exactly the same conditions. The method is carried out as follows: Phenol solutions of known strength are used. The cultures are grown in a standard medium, from which transplants are made every twenty-four hours. The inoculations are made with platinum loops of standard size. Usually about four different dilutions of the phenol and the disinfectant to be tested are employed. Five ccs of these dilutions are placed in sterile tubes, to which are added at one-half minute intervals one drop per cc of solution of the culture of the test organism. At the end of two and one-half minutes a loopful of each of the mixtures is transferred to a test tube containing 5 ccs of a standard culture medium, an interval of half a minute being thus allowed for taking the samples from the different solutions. These transfers are repeated at the end of 5, 7½, 10, 12½ and 15 minutes. The inoculated tubes are then incubated for 48 hours, after which the comparisons are made between the relative strengths of phenol and of the disinfectant, at which growth is prevented. The modification of this method by Anderson and McClintic (1), which is known as the "Public Health Laboratory Method," consists in comparing the effect of the phenol and disinfectant at 2½ and 15 minutes' exposure, and in extending the period of exposure to thirty minutes instead of fifteen. The number of dilutions employed are also much greater. The Lancet (2) method differs from the "Public Health Laboratory Method," which is a modification of it, chiefly in the method of making the transfers. In the Lancet method spoons are used instead of platinum loops for transferring the treated culture to the medium.

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(1) Journal of the Royal Sanitary Institute, Vol. XXIV, 1903 and 1904, pp. 424-435.

METHOD FOR PHENOL COEFFICIENT DETERMINATION:

The accuracy of these determinations depends largely upon the following factors: (1) Temperature of medication; (2) uniformity of age and general condition of the test organisms; (3) uniformity in the proportion of inoculating culture to disinfectant. The temperature at which the test organisms was exposed to the germicide was controlled in our experiments by means of a large bath, which was placed in a wooden box and packed in sawdust. The seeding tubes were submerged beneath the surface of the bath to a depth at which their contents would be entirely below the surface of the surrounding water. Through the top of the box, which was securely fastened, were holes for twenty-one tubes, a thermometer, and one for filling and siphoning off the water. The bath was made according to the description given in Bulletin 82 of the Hygienic Laboratory. It proved very satisfactory, and there was but little difficulty in maintaining the standard temperature at 20°C. throughout the experiments. For sterilizing the platinum loops a fan-tail gas burner was used and a wire rack to hold six of these loops, so that they could be flamed immediately after each transfer. A test tube rack of the required capacity was made from heavy timber by drilling holes of the proper diameter into it to the required depth. The seeding tubes were ¾″ in diameter and three inches long. The ones used in the "Public Health Laboratory" were one inch in diameter. Five ccs of the dilutions of the various germicides were placed in these tubes, after they had been sterilized in the autoclave. One-tenth cc of the inoculating culture was introduced into them and the mixture thoroughly shaken. The platinum loops for transferring the culture from the seeding tubes were 4 mm in diameter.

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(1) Hygienic Laboratory Bulletin No. 82.
(2) Lancet, London, Vol. 177, Nos. 4498, 4499, 4500.

The organism first selected for these experiments was *Bacillus vulgatus.* It was grown in a standard sucrose solution of the same formula as the one used by the author in the investigation of the species occurring in sugar ([1]). Only twenty-four hour cultures were used in the experiments and the incubation period of the tubes, to which the test culture was added, was forty-eight hours. Although it was especially desirable that the tests should be made on this species, there were several factors that militated against successful results. The species forms spores, which are intensely resistant to heat, and is one of the group causing deterioration of sugars. It was thought, therefore, that the results obtained upon its resistance to the different germicides would be especially interesting. It was found, however, to be practically impossible to secure concordant results from the use of this test organism in the experiments. At first the variations in the phenol coefficients in the different experiments were attributed to the influence of gum, which might occur in varying quantities in the different twenty-four hour cultures. The presence of this gum might tend to prevent the germicides from reaching the organism, and in this way the efficiency of the germicide might vary with the amount of gum in the solutions. Filtering off this gum by passing it through a sterile cotton filter, however, gave no better results. It was then discovered that the variations in the results were due to the fact that in some cases spores were present even in the twenty-four hour cultures, and as the strengths of the germicides were entirely insufficient for spore destruction, the germination of these spores and the subsequent growth of their vegetative forms would not be prevented by the highest concentrations of the germicides. The use of this species as a test organism was abandoned and a non-sporulating species substituted for it. The species then

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(1) Bulletin 146, La. Experiment Station.

selected was *Bacterium xylinum.* This species is almost invariably found in sugar cane juice, molasses and syrups, where it forms a thick gelatinous membrane known as chitine ([1]), which has been thoroughly studied by C. A. Browne. It belongs to the acetic ferment group of bacteria. The stock solutions of formaldehyde and phenol used in the experiments were accurately standardized at frequent intervals to determine their strength. The method used for the determination of formaldehyde was the modified hydrogen peroxid method, as given in the official methods ([2]) of the Bureau of Chemistry. For the standardization of the stock phenol solution the Meissenger and Vortmann ([3]) iodine method was employed.

TABLE XXII.

SHOWING PHENOL COEFFICIENT OF FORMALDEHYDE.
Proportion of Culture and Germicide, 0.1 c. c. to 5 c. c.
Temperature of Treatment, 20° C.
Culture used, *Bact. xylinum.*

Germicide	Strength	Time Culture Exposed to Action of Germicide					
		2½′	5′	7½′	10′	15′	30′
Formaldehyde	1:150	—	—	—			
	1:175	×	—	—			
	1:200	×	—	—	—	—	—
	1:250	×	×	—	—	—	—
	1:300	×	×	×	×	×	—
	1:400	×	×	×	×	×	—
	1:500	×	×	×	×	×	×
Phenol	1:125	—	—	—			
	1:150	—	—	—	—		
	1:175	×	—	—	—	—	—
	1:200	×	×	—	—	—	—
	1:250	×	×	×	×	—	—

× Indicates growth.
— Indicates no growth.

$$\text{Phenol Coefficient}\ \frac{\frac{150}{150}+\frac{250}{250}}{2}=1$$

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[1] The Chemistry of the Sugar Cane and Its Products in Louisiana. Bulletin 91, La. Expt. Station.

[2] Bulletin 107 (revised), p. 33, Bureau of Chemistry, U. S. Dept. Agriculture.

[3] Sutton's Volumetric Analysis, p. 395.

In the above experiment formaldehyde has a phenol coefficient of 1, which is much lower than was anticipated. We should have expected a coefficient of 3 to 3½, judging from the reputed antiseptic values of the two substances.

TABLE XXIII.

SHOWING PHENOL COEFFIENT OF FORMALDEHYDE.
Culture used, *Bact. xylinum.*
Temperature of Treatment, 20° C.
Proportion of Culture and Germicide, 0.1 c. c. to 5 c. c.

Germicide	Strength	Time Culture Exposed to Action of Germicide					
		2½′	5′	7½′	10′	15′	30′
Formaldehyde	1:150	—	—	—			
	1:175	×	—	—			
	1:200	×	×	—	—	—	—
	1:250	×	×	×	—	—	—
	1:300	×	×	×	×	×	—
	1:400	×	×	×	×	×	—
	1:500	×	×	×	×	×	—
Phenol	1:125	—	—	—			
	1:150	—	—	—	—		
	1:175	×	—	—	—	—	—
	1:200	×	—	—	—	—	—
	1:250	×	×	×	×	—	—
	1:300	×	×	×	×	×	—

× Indicates growth.
— Indicates no growth.
Phenol Coefficient $\frac{\frac{150}{150} + \frac{250}{250}}{2} = 1.$

The results shown in the above table are the same as obtained in the previous experiment.

Table XXIV.

SHOWING PHENOL COEFFICIENT OF FORMALDEHYDE.

Culture used, *Bact. xylinum.*
Temperature of Exposure, 20° C.
Proportion of Culture to Germicide, 1 c. c to 5 c. c.

Germicide	Strength	Time Culture Exposed to Action of Germicide					
		2½′	5′	7½′	10′	15′	30′
Formaldehyde	1:150	—	—	—			
	1:175	×	—	—	—		
	1:200	×	—	—	—	—	—
	1:250	×	×	×	×	—	—
	1:300	×	×	×	×	×	—
	1:400	×	×	×	×	×	×
	1:500	×	×	×	×	×	×
Phenol	1:125	—	—	—			
	1:150	—	—	—	—		
	1:175	—	—	—	—	—	—
	1:200	×	×	—	—	—	—
	1:250	×	×	×	×	×	×

× Indicates growth.
— Indicates no growth.

$$\text{Phenol Coefficient} — \frac{\frac{150}{175} + \frac{250}{200}}{2} = 1.053$$

In this experiment formaldehyde has a phenol coefficient of slightly over 1.

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TABLE XXV.

SHOWING PHENOL COEFFICIENT OF FORMALDEHYDE.

Germicide	Strength	Time Culture Exposed to Action of Germicide					
		2½′	5′	7½′	10′	15′	30′
Formaldehyde	1:150	—	—	—			
	1:175	—	—	—	—		
	1:200	—	—	—	—	—	—
	1:250	×	—	—	—	—	—
	1:300	×	×	×	×	×	—
	1:400	×	×	×	×	×	—
	1:500	×	×	×	×	×	—
Phenol	1:125	—	—	—			
	1:130	—	—	—	—		
	1:175	×	—	—	—	—	—
	1:200	×	×	—	—	—	
	1:250	×	×	×	×	—	—
	1:300	×	×	×	×	×	—

× Indicates growth.
— Indicates no growth.

$$\text{Phenol Coefficient} = \frac{\frac{200}{150} + \frac{250}{250}}{2} = 1.166$$

From these experiments it appears that formaldehyde has a Phenol Coefficient upon *Bacterium xylinum* of 1.05.

The comparative germicidal efficiency of formaldehyde, as shown in these experiments, is much lower than one would have expected. It is true that we have no data on the specific action of this germicide upon the species of bacteria that we employed as a test organism. Since different species of bacteria vary widely in their resistance to different germicides, we need not necessarily expect our results to agree with those derived from investigations on the action of formaldehyde on other species. The literature on the germicidal efficiency of formaldehyde shows a number of apparent contradictions. Sternberg (1) quotes Pottevin, who states that formaldehyde in the proportion of

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(1) Text Book of Bacteriology.

1:1000 kills vegetative forms of bacteria in from fifteen minutes to several hours. The same author also quotes Vanderlinden and de Buck, who cite instances where formaldehyde was decidedly inferior to corresponding solutions of phenol. When tested upon *Bacillus coli communis* and *Staphylococcuc pyogenes aureus,* a five per cent solution of formaldehyde failed to destroy them. Ordinarily, however, formaldehyde is believed to be efficient as an antiseptic in the proportions of 1:1000, while the antiseptic strength of phenol is 1:333. There is another factor which probably tended to lower the relative germicidal efficiency of formaldehyde in our experiments. We have already observed that the efficiency of formaldehyde is greatly increased at elevated temperatures. The temperature of 20°C., at which the phenol coefficient was derived, was comparatively low. It is very probable that phenol is relatively more active at this temperature than formaldehyde. Our previous experiments on the influence of temperature upon the efficiency of the various germicides showed that they were not all affected alike by elevations in temperature.

THE PHENOL COEFFICIENT OF FORMALDEHYDE AS AFFECTED BY THE PRESENCE OF ORGANIC MATTER.

We have already referred to the fact that the efficiency of many germicides is greatly impaired in the presence of organic matter. It is on account of the fact that the germicidal action of phenol is not affected by these conditions that it is accepted as a standard of comparisons in experiments of this kind. In order to determine the phenol coefficient of formaldehyde under conditions that would be more comparable with sugar house conditions, experiments were conducted upon the action of formaldehyde on *Bacterium xylinum* in the presence of cane syrup. Anderson and McClintic ([1]) used a peptone gelatine solution in distilled water in their tests. For our purpose the influence of sugar house products upon the efficiency of germicides is of more practical significance.

TABLE XXVI.

SHOWING INFLUENCE OF ORGANIC MATTER UPON GERMICIDAL EFFICIENCY OF FORMALDEHYDE.

Germicide	Strength	Time Culture Exposed to Action of Germicide					
		2½′	5′	7½′	10′	15′	30′
Formaldehyde	1:125	—	—	—			
	1:150	×	—	—			
	1:175	×	×	—	—		
	1:200	×	×	×	—	—	—
	1:250	×	×	×	×	×	—
	1:300	×	×	×	×	×	—
	1:400	×	×	×	×	×	—
	1:500	×	×	×	×	×	×
Phenol	1:200	—	—	—			
	1:225	×	×	—	—		
	1:250	×	×	—	—	—	—
	1:300	×	×	×	—	—	—
	1:350	×	×	×	×	—	—

× Indicates growth.
— Indicates no growth.

$$\text{Phenol Coefficient} = \frac{\frac{125}{200} + \frac{200}{350}}{2} = .5985$$

Table XXVII.

SHOWING INFLUENCE OF ORGANIC MATTER UPON THE GERMICIDAL EFFICIENCY OF FORMALDEHYDE.

Germicide	Strength	Time Culture Exposed to Action of Germicide					
		2½′	5′	7½′	10′	15′	30′
Formaldehyde	1:125	—	—	—			
	1:150	×	—	—	—	—	—
	1:175	×	—	—	—	—	—
	1:200	×	×	×	—	—	—
	1:250	×	×	×	×	×	×
	1:300	×	×	×	×	×	×
	1:400	×	×	×	×	×	×
Phenol	1:200	—	—	—			
	1:225	×	—	—	—		
	1:250	×	—	—	—	—	—
	1:300	×	×	—	—	—	—
	1:350	×	×	×	×	—	—

× Indicates growth.
— Indicates no growth.

$$\text{Phenol Coefficient} = \frac{\frac{125}{250} + \frac{200}{350}}{2} = \frac{.625 + .572}{2} = .5985$$

The method employed in these experiments was as follows: Solutions of formaldehyde and phenol were prepared in such strengths that by the addition of 1 cc of a sterile syrup solution they would represent the same dilutions as employed in the previous experiments. For example, if it were desired to test formaldehyde in the proportion of 1:100 a stock solution of 1:80 was prepared. Four ccs of this solution when diluted by 1 cc of the syrup solution would represent a dilution of 1:100. The method of conducting the experiment was, with this exception, exactly the same as that above described. The syrup solution was first sterilized in an Arnold sterilizer for fifteen minutes on three successive days. It was delivered into the seeding tubes by sterile pipettes, after 4 ccs of the different germicides had first been introduced.

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(1) Loc. cit.

Table XXVIII.

SHOWING INFLUENCE OF ORGANIC MATTER UPON GERMICIDAL EFFICIENCY OF FORMALDEHYDE.

Germicide	Strength	Time Culture Exposed to Action of Germicide					
		2½′	5′	7½′	10′	15′	30′
Formaldehyde	1:125	—	—	—			
	1:150	×	×	—	—		
	1:175	×	×	—	—	—	—
	1:200	×	×	×	×	×	×
	1:250	×	×	×	×	×	×
	1:300	×	×	×	×	×	×
	1:400	×	×	×	×	×	×
Phenol	1:200	—	—	—			
	1:225	×	—	=	—		
	1:250	×	×	—	—	—	—
	1:300	×	×	×	×	—	—
	1:350	×	×	×	×	×	—

× Indicates growth.
— Indicates no growth.

$$\text{Phenol Coefficient} = \frac{\frac{125}{200} + \frac{175}{300}}{2} = 6045$$

An average of these three experiments gives formaldehyde a coefficient of .6015, which is considerably lower than the result obtained in the experiments without organic matter. This decrease in its phenol coefficient appears to be due to an increase in the germicidal power of phenol, as well as to a slight decrease in the germicidal efficiency of formaldehyde. In the two series of the experiments the average strengths of formaldehyde and phenol required to prevent growth in a 2½ and 15 minute exposure was as follows:

Germicide	Without Organic Matter		With Organic Matter	
	2½′	15′	2½′	15′
Formaldehyde	1:150	1:250	1:175	1:191
Phenol	1:158	1:233	1:200	1:333

The decrease in the germicidal efficiency of formaldehyde in the presence of cane syrup may be explained by the results of the previous experiments on sugars and fermenting syrup solutions. In these experiments the low efficiency of formaldehyde was attributed to the formation of some combinations between the formaldehyde and the sugars present. The increase in the germicidal action of phenol in the presence of syrup is not so easily explained. That it was not merely due to changes in the composition of the solutions was proven by repeated standardization of the stock solutions. There is a law of germicidal action which may account for this increased efficiency of phenol under these conditions. This law is known as "The Choice of Solvents" (1). That is to say, the action of a germicide is increased in the proportion in which it is more soluble within the cells of the microorganisms than outside. A germicide has the choice, so to speak, of penetrating the cell or remaining in solution on the outside, and the former course will be followed to the extent in which it offers the lesser resistance. For example, the addition of salt to an aqueous phenol solution increases its germicidal action because it is less soluble in the salt solution. There are cases, however, where the germicidal action depends upon dissociation, in which the above principle does not apply. For example, the addition of sodium chloride decreases the germicidal action of bichloride of mercury because it decreases its dissociation.

It seems probable that the increased efficiency of phenol in the experiments with syrup was due to its lower solubility in that solution.

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(1) Bennecke Bau und Leben der Bakterien.

THE PHENOL COEFFICIENT OF CHLORIDE OF LIME.

The experiments upon the comparative efficiency of phenol and chloride of lime brought out a very surprising fact. It was found that *Bacterium xylinum* is intensely sensitive to chlorine. The stock solutions of chloride of lime were made and standardized by Penot's method (1), which consists in titrating it with an arsenious solution. At first the dilutions were from 1 : 50 to

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(1) Sutton's Volumetric Analysis, p. 174.

1:150, but as these seemed too strong and no growth occurred in any of the transfers made from them the dilutions were increased. After experimenting with dilutions up to 1:1000 without obtaining any positive results, it was thought that there must have been some defect in the method. The test culture was in good condition, as shown by the fact that the growth in the phenol transfer tubes corresponded with those obtained in previous experiments, and also by the normal rate of growth in the control transfers. The two possible explanations of these apparently inconsistent results were as follows: (1) The amount of chlorine carried into the culture tubes from the tube containing the mixture of chloride of lime and test organism might have been sufficient to have prevented the growth of the bacteria, if they had survived the original exposure. (2) The dilution of the culture from .1 cc to 5 cc might have been so great that none of the bacteria were carried over by the platinum loop into the tubes of culture medium. That the first of these theories could not have accounted for the negative results was proven by the fact that the addition of a platinum loopful of the strongest of the chloride of lime solutions to tubes containing sucrose solution which had been inoculated in the usual way, did not prevent growth, nor was the degree of dilution so great that the transfers from the seeding tubes contained none of the bacteria, because transfers of the same amount of culture from a tube that had been similarly diluted with water always resulted in growth.

TABLE XXIX.

SHOWING PHENOL COEFFICIENT OF CHLORIDE OF LIME.

Germicide	Strength	Time Culture Exposed to Action of Germicide					
		2½′	5′	7½′	10′	15′	30′
Chloride of Lime	1:40,000	—	—	—			
	1:42,500	×	×	—	—		
	1:45,000	×	×	×	—	—	—
	1:50,000	×	×	×	×	×	×
	1:51,000	×	×	×	×	×	×
Phenol	1:150	—	—	—			
	1:175	—	—	—	—		
	1:200	×	×	—	—	—	—
	1:250	×	×	×	×	—	—
	1:300	×	×	×	×	×	—

× Indicates growth.
— Indicates no growth.

Phenol Coefficient $$\frac{\frac{40,000}{175} + \frac{45,000}{250}}{2} = 204$$

The results of the above table show that chloride of lime has a very powerful germicidal action upon *Bacterium xylinum.* The phenol coefficients of the other germicides were not determined. It is probable that of all the other germicides used in the previous experiments only the ammonium fluoride would have shown a positive phenol coefficient, and it is likely that it could not have been used in strengths sufficient to prevent growth in an exposure of 2½ minutes. Furthermore, the results obtained from the use of a single species of bacteria is not sufficiently representative of the relative efficiencies of the germicides under sugar factory conditions to warrant a continuance of these experiments.

Chapter V.

THE RELATIVE COST OF THE VARIOUS GERMICIDES PER UNIT OF GERMICIDAL EFFICIENCY.

In order to derive an adequate idea of the relative value, for sugar factory use, of the various germicides used in the preceding experiments, it is necessary to take their respective cost into consideration. A germicide which costs one dollar per gallon may be cheaper than another which costs only fifty cents, if the germicidal efficiency of the former is three times as great. It is essential to a fair comparison of the value of the germicides that we determine from their relative cost and their germicidal efficiency their relative cost per unit of effectiveness. The cost of the germicides per pound was obtained from catalogue prices. The price given for formaldehyde is that which is quoted in the catalogue of Perth Amboy Company for barrel lots. In smaller quantities it costs 12 cents per pound. It is probable that many of the other germicides might be obtained at lower prices than those taken as our basis of comparison. These prices are given in the catalogues and apply to small lots rather than purchases in large bulk. The price in each case applies to grades for technical purposes and not to the C. P. quality. The price given for quicklime is for barrel lots.

The cost per unit of efficiency is derived by assuming that of formaldehyde, which is taken as the standard of comparison, to be 1 in every case. Thus we have the formula: (1)

$$\begin{array}{ll} \text{Cost of formaldehyde per pound} & \\ \hline \text{Cost per pound of germicide} & = (\text{cost ratio}) \div \\ \hline \text{Coefficient of formaldehyde} & (= 1) \\ \text{Ccefficient of germicide} & \end{array}$$

This formula is used in determining the cost per unit of efficiency of disinfectants by the phenol standardization method, but it is just as suitable where formaldehyde is used as the standard, when the proper substitutions are made.

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(1) Hygienic Laboratory Bulletin No. 82.

Table XXX.

SHOWING RELATIVE COST OF GERMICIDES PER UNIT OF GERMICIDAL EFFICIENCY.

	Cost per Pound	At Room Temp.	Experiments on Cane Juice					Fermenting Syrup Sol.	Deteriorated Sugar Sol.
			At Temp. for Max. Form. Coefficient	Ferment Juice		Bucket Experiments			
				Slightly Fermented	Badly Fermented	Gummy Juice	Badly Fermented Juice		
Formaldehyde	9¢	1	1	1	1	1	1	1	1
Chloride of Lime	6¢	1.77	1.67	.537	.0579	.257	.320	2.25	3.01
Ammonium Fluoride	60¢	55.6	29.6	4.14	18.8	61.1	3.66	61.7	41.9
Bisulphite of Soda	25¢	140.0	140.0	245	16.7	30.3	14.7	308	174
Bisulphite of Lime	35¢	.....	.....	173	40.6	.....	.....	45.2	730
Sodium Fluoride	25¢	96.7	70.5	128	150.0	127	7.44	280	48.7
Milk of Lime	1¢	1.33	0.175	0.411	.125	.0966	.197	1.45	1.39

A study of the above table leads to the following conclusions:

(1) At the prices upon which these calculations were based only two of the germicides have as low a cost per unit of efficiency as formaldehyde.

(2) Formaldehyde is superior to any of the germicides for disinfecting syrup tanks or in removing the microorganisms causing deterioration of sugars.

(3) For cleaning tanks containing fermented juices, either chloride of lime or milk of lime can be substituted for formaldehyde at a reduction of cost per unit of efficiency.

(4) In cases where the efficiency of the germicide involves any great penetrating power, as, for example, where gums are to be removed, chloride of lime is a more economical germicide than formaldehyde.

(5) Since the efficiency of all germicides is greater at elevated temperatures, they should always be applied hot in cleaning sugar factories.

(6) The comparatively high germicidal efficiency of milk of lime, combined with its very low cost, and the fact that it is always on hand in sugar factories, renders it particularly suitable as a sugar factory germicide.

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Baton Rouge

Protecting Cabbage and Cauliflower From Attacks by Worms.

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PROTECTING CABBAGE AND CAULIFLOWER FROM ATTACKS BY WORMS.

By E. S. TUCKER, Associate Entomologist.

By way of introduction, a few words on the status of the above commodities are given to impress the point that their value as assets of the trucking resources of this State depends considerably upon the aid of suitable practices for combating the insect enemies. Although cabbage and cauliflower are rated among the important truck products of Louisiana, the prospect of these two edibles being grown in far greater quantities seems to be well assured.

As one of the principal vegetables that has all along occupied a place in home gardens, cabbage has shown its adaptability to thrive in nearly every kind of soil within the State. Of late years, however, it has become cultivated to a steadily increased extent as a field crop in many sections where facilities permit it to be marketed advantageously, either for local consumption or through shipments to northern centers of trade.

Since the growing of cauliflower has been satisfactorily demonstrated within recent years by the horticulturist of the Agricultural Experiment Station at Baton Rouge, numbers of truckers and farmers have taken interest in it and made similar plantings with profit, besides supplying their tables at first hand with a particularly savory dish that the product affords. The success attending the cultivation of these crops has opened new possibilities for the utilization of silt lands, of which the lower alluvial ones under certain conditions are found to be especially favorable for the production of this choice vegetable. With the encouraging results so far obtained by producing and marketing cauliflower in amounts up to full car lots under organized management by the growers, its culture is apparently destined to attain to the distinction of a resourceful enterprise.

FIGHTING THE INJURIOUS INSECTS PAYS.

All truck plants on account of their succulent growth are particularly subject to depredations by insects, and cabbage and cauliflower afford no exception. Both of these plants, therefore, have their enemies. Owing to the close relationship of the two vegetables, they are attacked generally by the same kinds of pests. each of which is about as fond of one plant as of the other. Unless effective means are employed to check these attacks. the plants if they escape being killed at the outset will at some time, mainly during the warm season, inevitably suffer material injury. After the growth has once advanced to a hardy stage, the harm then done to it causes a tardy and often imperfect development of the heads.

In most cases, these troubles can be largely avoided by simple protective measures if they are exercised at the right time. Therefore, when the adversities can be greatly prevented by precautionary action at little effort and expense, the benefits to be gained through the approved practices towards securing full crops of choice quality is reasonably certain to bring a profitable reward. The advantage of getting a yield of greater value through perfect growth in a shorter time by judicious control of pests, than would be possible without any protection being given to the crops, leaves no ground for dispute nor excuse for neglect to fortify the plants against serious ravages.

Different ways of defense have proved their worth for suppression of the various sorts of harmful insects at a comparatively slight cost. Yet, no single method is sufficient for universal use. The remedy must be chosen with regard to its property of acting on a pest in disposition with the nature of it. Consequently, an understanding of the life history, habits and structure of the different groups of insects is indispensable for selection of the right course to pursue for combating them.

While many kinds of insect foes attack the growing plants, the most destructive ones appear in the form of worms, which are more properly called larvæ or caterpillars. In due course, they change into certain kinds of butterflies and moths. A brief description and account of the prevalent species will help towards their recognition in the field.

THE IMPORTED CABBAGE WORM.

(*Pontia rapae* L.)

The most common insect enemy and therefore the worst one that feeds on cabbage and related plants throughout our country and also in Canada does its destructive work in the form of a velvety green worm. This wormy-looking creature is not a true worm, but is really the larva or caterpillar of a white butterfly. The latter is the mature stage or parent, which has a wing expanse of nearly two inches, being marked with black tips and two conspicuous black spots on each fore wing of the female, while the male has but a single spot instead. Both sexes have a corresponding smaller black spot near the upper extremity of the hind wings.

Soon after mating, the female commences to deposit pale yellowish and rather elongate eggs with ribbed sides, attaching them singly to the outer surface of leaves in preference to the inner side, except on very young plants. A keen observer may distinguish the egg by the naked eye.

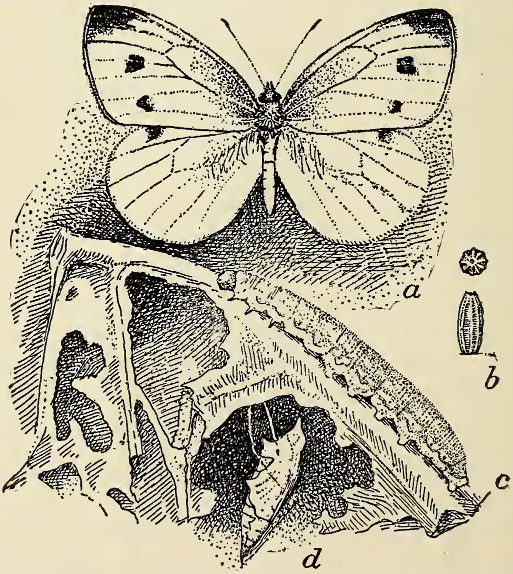

Fig. 1.—The imported cabbage worm (*Pontia rapae*): a, female butterfly; *b*, above, egg as seen from outer end; below, as seen from side; *c*, larva in natural position on cabbage leaf; *d*, suspended chrysalis—*c*, slightly enlarged; *b*, more enlarged. (After Chittenden, Cir. 60, Bu. Ent., U. S. D. A.)

The egg hatches in from four to eight days, according to weather conditions, and produces the young caterpillar which feeds voraciously on the foliage of cabbage or cauliflower as the case may be, often attacking turnips, collard, radishes, mustard and horseradish. Its work is characterized by large ragged gaps in the leaves, and on forming cabbage, the worm may eat holes into the head itself. Besides its repulsive appearance, the smears of excrement which it drops on the growth makes its presence very objectionable on plants desired for food.

Under a magnifier, it shows a faint yellow stripe along the middle of the back, a row of yellow spots on each side, and fine black dots on the upper part of the body. It becomes full grown in about ten days or two weeks, reaching a length of one and one-quarter inches. Then it spins a silky girdle and swings itself from a leaf or stalk, and usually within a day, transforms into a chrysalis of the color of the plant upon which it may be suspended, though shrinking to less than an inch in length. In this stage, it resembles a bit of rolled leaf and remains apparently lifeless for a week or two in summer time, but in northern climates, it passes the winter in this condition.

From the chrysalis, the adult butterfly emerges and another life cycle is then repeated. Under different temperatures, the entire period of development varies from twenty-two days to five weeks. As many as five or six generations probably occur during the year in Louisiana, where, as in other gulf states, the butterfly may frequently be seen through the winter.

Like many other serious pests, this insect is an introduced one, having come from Europe and invaded our continent. Curiously enough, its competition with our native or southern cabbage butterfly has caused the latter to give way before the superior foreign foe. Tiny wasp-like parasites attack and kill many larvæ and chrysalids, and therefore do a beneficial work. But means of checking this species sufficiently depends on direct treatment as explained farther along. Picking and destroying the worms by hand is practical in small plats. The custom of leaving stalks in the field after cutting of plants is a grave mistake. All remnants of food plants should be gathered and destroyed.

RECORDS APPLYING TO THE IMPORTED CABBAGE WORM.

From the following complaints of growers in Louisiana, together with observations made by the writer, many particulars of interest and value may be gleaned. The inquiries show that farmers and truck raisers are earnestly seeking information.

Elton, Jefferson Davis Parish, April 27, 1913.—In asking what could be done to keep the worms from harvesting a crop of cabbages which were nearly grown, the applicant intimated that the yield was threatened and the results of his labor might be lost.

Baton Rouge, September 22, 1913.—The imported cabbage worm had proved to be the most destructive insect enemy of cauliflower plants which were then being grown on the Experiment Farm. Its control, however, had just been accomplished by use of powdered arsenate of lead shaken onto the plants from bags made of cheesecloth. At this time, scarcely any of the worms remained alive, but numbers of dead and shriveled bodies hung to the leaves of nearly every plant. As many as two to four lifeless examples appeared on single leaves. Owing to a period of rainy weather, the poison had to be applied frequently, or on an average of about twice per week. From four to ten pounds of the poison, depending on the size of the growth for treatment in different plats with early to late plantings, had been used for each application per acre. Since prevailing heavy rains had often washed off the powder soon after being dusted on the plants, many worms had failed to get a dose of poison for some time and meanwhile had made considerable headway in eating the foliage. Nevertheless, persistence in treating with the poison saved the crop from serious depredations even under the excessive rainy conditions.

LaPlace, St. John the Baptist Parish, March 20, 1914.—Inquirer expressed anxiety concerning the advances of worms in riddling his cabbage at this early date in the season.

Garland, St. Landry Parish, March 28, 1914.—While signifying a desire to know how to fight the insects attacking his cabbage, the questioner furnished some small rove beetles in the belief that injuries to the leaves and stalks of the plants had been committed by these forms. The leaves, however, gave strong evidence of having been fed upon by worms. Whether the little beetles had done any harm or merely acted as scavengers could not be told, as they usually do not feed on growing vegetation. At least in considering the diminutive size of the beetles, they hardly seemed capable of making the large holes in the leaves or eating out such large cavities as appeared in the stalks. Advice was given to apply an arsenical poison as recommended for killing worms.

Trout, La Salle Parish, June 16, 1914.—Correspondent stated that he had grown a fine patch of cabbage and collard, but the plants were then being literally devoured by small green worms, for which he could find no remedy. Although he had tried picking off the worms, yet on the next day they seemed to be more numerous than ever. To succeed by this method, one should persist in picking regularly. In desiring to know of better means for destroying the pest, he want to be sure and not poison the cabbage so as to make it dangerous to be eaten. On this point, he was assured of no danger with proper care.

Baton Rouge, September 26, 1914.—An appeal for prompt information giving a formula for spraying cabbage to keep insect pests, among which the cabbage worm was mentioned, from ruining the plants, came from the Seventh Ward of the parish.

Napoleonville, Assumption Parish, October 8, 1914.—The question was asked if arsenate of lead could be added to kerosene emulsion in order to spray for both cabbage worms and plant lice at the same time. In reply, the party was informed that kerosene emulsion can not be successfully combined with stomach poisons of an arsenical nature. However, a combination spray effective on both worms and lice may be made with nicotine solution, such as "Black Leaf 40", 2 tablespoonfuls; soap, 4 ounces; Paris green, 1 ounce; and water, 4 gallons. In place of Paris green, lead arsenate at half the quantity would probably be safer to plants and more powerful against chewing insects if used in the mixture.

Baton Rouge, April 6, 1915.—The beginning of ravages for the season showed that the worms had appeared in considerable force on cabbage at this time.

Oxford, De Soto Parish, April 21, 1915.—A request for a pamphlet on suppression of the green cabbage worm has impressed the necessity of preparing the present publication. In replies by letter, time does not permit of giving more than the shortest advice that will answer the purpose.

Bernice, Union Parish, April 23, 1915.—A teacher stated that worms were doing much damage to the cabbage being grown in home gardens. But no efforts to his knowledge had been spent towards protecting the crop against the depredations. Farmers seemed to be afraid to apply poison to the plants, thinking that the heads would be made unsafe for consumption. As soon as they come to understand that practically no risk is involved, they will doubtlessly adopt the methods of control and thus prevent a great loss of products.

Coushatta, Red River Parish, May 5, 1915.—A farmer who applied for advice concerning remedies for insect pests on different kinds of truck plants referred to worms on cabbage as the first enemy. The treatments with arsenicals as prescribed for this foe should be sufficient for most other insects which feed by chewing the leaves or exposed parts of plants.

THE SOUTHERN CABBAGE WORM.

(*Pontia protodice* Boisd.)

This worm differs but little in color and markings from its imported competitor, which has largely taken its place. While the life history and habits of this native species are quite similar to the foreign enemy, it has not been known to range very far north. Hence it is named for its southern preferences. Both sexes of the butterfly have white wings with several black spots on the primaries, forming broad oblique dashes on the interior area, though the female is more intensely marked and has zigzag bars of grayish tint on the hind wings. The expanse is slightly larger than for the immigrant invader. Occasionally, a specimen or two may be seen, but the insect has become too scarce to be reckoned as a pest for many years.

THE LARGE CABBAGE BUTTERFLY.

(*Pontia monuste* L.)

With a spread of wings from two to three inches, this white butterfly ranks as the largest of the cabbage insects. Yet it is not very common in Louisiana, although being partial to the southern states and tropical regions. Its larva is lemon-yellow in color and measures about one and one-half inches in length when full grown. Only one complaint in which this species seemed to be concerned has come to attention. The record follows:

Rayne, Acadia Parish, October 14, 1914.—Sender forwarded four caterpillars with letter saying that they were destroying his young mustard. Attempts to rear the examples resulted in noting the pupation of one on the 16th, two days after the sending, but mold attacked and killed the chrysalis.

THE CABBAGE LOOPER.

(*Autographa brassicae* Riley.)

Next to the imported cabbage worm, the looper often appears in destructive numbers, attacking cruciferous plants and also other kinds less frequently. This caterpillar is easily recognized by its habit of crawling with a looping motion. Among the individuals, particularly of different ages, the color varies between pale and dark green; but the body is striped with whitish lines, although these markings sometimes disappear at full growth of the larva. It attains to an inch in length. As it seeks the under sides of leaves, it is not apt to be reached with poisons unless they are carefully applied.

When ready to transform, the looper covers itself with a thin webby cocoon that is generally attached to a side of a leaf. Here it changes to a pupa having a length of about three-quarters of an inch, and remains quiescent while development goes on until a perfect moth is formed. At last the adult issues forth from the old integument and escapes through the cocoon. With wings expanded, the fore pair reaches more than an inch from tip to tip. The upper side of these wings has a dull brownish cast with fine mottlings. Then comes the laying of eggs of a silvery luster and almost globular form, which in turn hatch into another brood of the looper caterpillars ready for their raids.

This pest rather superseded the imported worm in feeding on cauliflower plants during the fall of 1914 at Baton Rouge, and was observed later, on November 9, to be the principal foe on cabbage and turnips growing near Alexandria, Rapides parish.

THE CROSS-STRIPED CABBAGE WORM.

(*Evergestis rimosalis* Guen.)

The cross-striped cabbage worm is so named on account of the many transverse black lines which mark its body. It is an ordinary pest, but has not been observed in great abundance. This fact and its medium size has apparently drawn little attention to it. The full-grown larva slightly exceeds five-eighths of an inch in length, and the moth has a wing expanse of an inch. Like other insects of its order, it passes through the four stages, egg, larva, pupa and adult, for complete development. Pupation is effected under the surface of the ground, in a cocoon constructed of particles of earth.

The accompanying reports give some idea of the occurrence of the insect on two occasions.

Baton Rouge, September 22, 1913.—A few caterpillars were found on cauliflower plants, where they had fed in the curls of the tender middle leaves.

Port Hudson, East Baton Rouge Parish, November 19, 1913.—Attention having been drawn to perforated leaves of young turnips, examination revealed a small caterpillar at work.

HOW TO COMBAT THE PESTS.

One important point to be remembered is that clean culture should be practiced. Directly after harvesting a crop, remove and burn all old stalks, worthless plants and stray leaves. Except in very small plantings, hand-picking of worms and destroying them becomes a tedious task. In home garden patches, however, hot water can be employed to scald the caterpillars on cabbage and cauliflower plants, or such as have hardy leaves, but care should be taken not to apply water any hotter than 135 degrees. At this temperature, it will not harm coarse foliage.

Pyrethrum powder is non-poisonous to human beings or live stock, but aside from its rather high cost, it is liable to stain the vegetation; and at the best, only young worms succumb to it. Hellebore is somewhat effective, yet it falls short of the action of arsenicals. Therefore, the use of the latter poisons affords the most reliable and economical means of combating the pests.

DIRECTIONS FOR POISONING WORMS ON LEAVES.

Suppression of all such insect pests, which feed by chewing the leaves or exposed parts of plants, may be quite readily accomplished by either spraying or dusting the growth with poison in any way hereby prescribed.

For wet treatment when a sprayer is obtainable, use lead arsenate paste at two pounds to fifty gallons of water, with addition of two or three pounds of stone lime, or else take Paris green at one pound to one hundred gallons of water, with same amount of lime as previously mentioned. Apply thoroughly with a good sprayer about every week or ten days.

Dusting plants with dry powdered arsenate of lead mixed equally with air-slacked lime, or else with a mixture of Paris green with fifteen times its bulk of the lime dust, either to be applied by shaking from cheesecloth bags, or by means of a blow-gun, is the simplest method, though it is not as effective as spraying. Put mixture on plants when they are moist with dew to make powder stick and last to best advantage, and use persistently as needed. Remember that the lime should never be omitted, or burning of foliage will be liable to result.

Arsenate of lead at full strength is generally harmless to old plants but not to young and tender growth. To avoid risk of injury by it in any case, it is safer when mixed with an equal amount of lime.

Laborers who handle the poisonous materials should exercise care, and when engaged in dusting, precaution needs to be taken against inhaling the powder. Choose a position with relation to the direction of the wind when at work so that the dust will not blow into the face.

NOTES ON TREATMENT WITH POISON.

Too much fear is commonly entertained that the application of poison on vegetables will make them dangerous for eating purposes. A striking instance came to notice in a home garden near Gueydan, Vermilion parish, on June 12, 1913, where the worms had been allowed to depredate unmolested on the entire patch of cabbage until none of it appeared to be fit for eating at all, yet the owner would not believe that poison could be used without danger to the family should the members eat such treated vegetables.

In fact, the usual poisonous dose for the insects is too slight to have any effect on persons, while after about a week from the application, it becomes obliterated from the plants, even wearing off in dry weather. Not a single case of human poisoning has ever been traced to the eating of treated vegetables, even after fresh applications. By the customary removal of the outer leaves, and further by careful washing, the product can be made perfectly safe for the table. All objections can thus be overcome. One authority states that a person would have to eat as many as twenty-eight cabbage heads in one meal to get any ill effects due to an ordinary treatment by poison.

While venturing the opinion that extra heavy applications of arsenicals might be sufficient to poison rabbits, for example, yet ordinary treatments must be regarded as being comparatively safe to all creatures except the small chewing pests. In connection with this subject, the following inquiry and remarks are introduced here.

Talisheek, St. Tammany Parish, March 14, 1915. — Grower wanted to know how to apply Paris green to truck plants, including cabbage, so as to poison rabbits, stating that he had known of its being done, but yet was afraid of killing the vegetables. He was advised to try powdered arsenate of lead, since Paris green is liable to burn foliage unless mixed with something to neutralize, as with fifteen or twenty times its bulk of lime dust, which will reduce the strength of the poison. On rank growth, as of cabbage, the powdered arsenate of lead may be dusted on at full strength without materially endangering the plants, but leaves should be kept heavily coated with it by daily applications until rabbits get enough of a dose to poison them. Of course, such treated plants which are wanted for the table should be very carefully washed when gathered. As to the value of this method of dealing with rabbits, however, no definite information is at hand.

In another case relating to domestic consumption, the party made known his alarm by writing an urgent letter beseeking advice in the matter as here presented.

Calhoun, Ouachita Parish, May 8, 1915.—Having put Paris green on his cabbages while they were heading, to kill worms, the owner desired to learn immediately if the vegetables would be dangerous to eat. As two showers of rain had fallen since the time of treatment, he feared that some poison had become washed into the heads, and asked if boiling the vegetables would do away with the danger. While only a light application had been made on both cabbage and collard, he supposed that the rains had completely washed off the poison from the latter plants. Assurance was given him that danger from use of arsenicals is very slight. The fact was pointed out that the dusting of cabbage and other vegetables grown on the State Experiment Farms, repeatedly with pure arsenate of lead, is a regular practice. The outer leaves of cabbage largely protect the head, and this loose growth is always removed when the head is prepared for eating. Then the head developes from the interior and applications cannot penetrate it. Besides, poison seldom remains on plants longer than a week or ten days even in dry weather, and a rain is apt to obliterate all traces at any time when it falls. To be perfectly safe, heads should be washed well if they are to be eaten raw, especially soon after a treatment, and boiling carried through a preliminary change of water ought to give absolute security to the prepared product.

FIGURES ON COST OF TREATING CAULIFLOWER CROPS.

According to the horticulturist of the Experiment Station at Baton Rouge, the cost of arsenate of lead for poisoning worms on cauliflower during the growing of a crop will amount to $5.00 and upward to $10.00 per acre, depending on the number of applications required to keep the plants treated under different weather conditions. For cabbage, the expense is estimated at about $5.00 per acre.

A planting company of Thibodaux, Lafourche parish, in reporting the cost of growing and crating a crop of cauliflower from three and one-half acres during the season of 1914 to 1915, at a total expense of $282.15, gave an item of $26.30 which was spent for ''arsenate of lead, etc.'', to poison worms. If the poison was purchased at quoted price of 18 cents per pound in 100-pound kegs, the quantity that was used figures out at 146 pounds, plus a small fractional excess. Considering that the outlay should include the freight charges and purchase of cloth for bags required to distribute the poison, then the actual amount of the powder which was applied in the field must have been a few pounds short of the above calculation. Nothing was said about the hire of labor in connection.

CUTWORMS.

Other wormy pests of cabbage and cauliflower, which deserve to be taken into account for this publication, are the cutworms. They are naked, plump-bodied, and dingy colored caterpillars that generally hide by day and forage at night. Though sometimes feeding on leaves, their habit of cutting off stems of young plants and working under cover of darkness makes them to be a dreaded foe in both gardens and fields. The different species need not be mentioned here, but they all produce dull marked moths or millers on reaching maturity. Transformation from larva to adult is effected in the ground.

Restraint of these enemies requires special measures, because the despoilers do not stay on plants. By plowing the land in fall or winter, many of the worms then hibernating in the soil will be killed. Clean culture is very advisable. Shingles or pieces of old boards when placed at intervals in the beds of young plants draw the pests into concealment beneath them, and a person making search regularly every morning may often find the larvæ hiding under such shelters and easily kill them on the spot. They may also be dug out of loose soil surrounding injured plants.

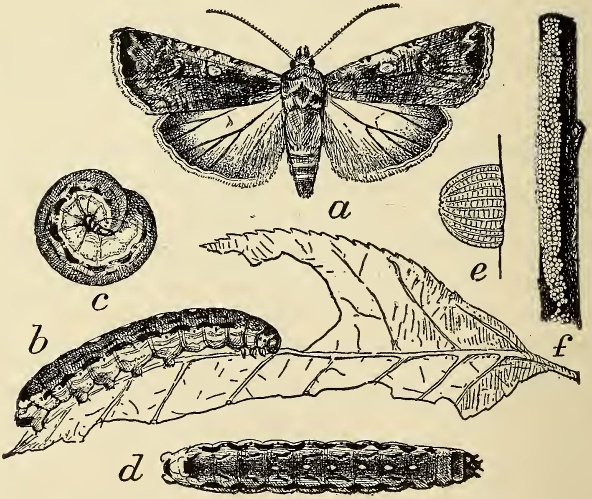

Fig. 20.—The variegated cutworm (*Peridromia saucia*): *a*, moth; *b*, normal form of larva, lateral view; *c*, same in curved position; *d*, dark form, dorsal view; *e*, egg from side, much enlarged; *f*, egg mass on twig. (After Howard, Bu. Ent., U. S. D. A.)

One of the most successful ways of checking cutworms is achieved by the use of poisoned bait or bran-mash. The bait is easily prepared by dipping freshly cut clover or some succulent vegetation in a solution of poison and scattering handfuls in places frequented by the worms so they will go to the lure. To make the other preparation, mix a small amount of white arsenic or Paris green in each pound of bran as will be needed for size of patch, sweeten by adding syrup or cheap molasses, and saturate it all with water. The juice of a lemon or orange also mixed in it is claimed to make it more savory to insects which operate just above the surface of the ground. Scatter this mixture where the worms occur, placing it out on the ground in the evening so it will be in readiness for the night and not dry too quickly. The caterpillars will then eat it in preference to attacking the plants, and consequently get a dose to cause their death. Care should be taken to keep poultry and other farm animals, as well as children, from having access to it.

Protecting plants with old tin cans having tops and bottoms removed, or by other devices, is frequently employed by gardeners. Tarred card-board disks, each being provided with a slit cut to the center so that the card can be slipped around the stem of the plant at the surface of the ground, are quite inexpensive and furnish a lasting guard against attacks.

REMARKS ON OCCURRENCE OF CUTWORMS.

The pest being of common occurrence and therefore generally expected to do some damage particularly to plantings early in spring, gardeners and other growers of truck have evidently become so accustomed to their work that the resulting losses are regarded as a regular event. Only a few reports have been received with appeals for directions to combat these enemies, and a review of these letters will give the localities, dates, and conditions as mentioned.

Oscar, Pointe Coupee Parish, Juy 25, 1913.—Resident begged to be informed of the best mixture for killing cutworms on cabbage and cauliflower, and also how to stop other insects from destroying the plants.

Cinclare, West Baton Rouge Parish, September 13, 1913.—In response to a personal request, information regarding the control of cutworms attacking cauliflower plants was promptly furnished for the benefit of a planting company that had undertaken to grow a crop of this kind of vegetable.

New Orleans, January 28, 1914.—Finding that cutworms had developed in new soil used for making a garden, the proprietor solicited a method to prevent the foes from destroying his radishes and lettuce.

Donaldsonville, Ascension Parish, April 22, 1914.—The query in this case related to trouble by something which ate young lettuce and green peas, devouring the leaves nearly to the ground. The statement that nothing could be seen on the plants led to the supposition that the growth had suffered attacks by cutworms operating at night.

SPECIMENS NEEDED.

Unless specimens are received with inquiries, the species of pest concerned in the cases can not be told with certainty, and only a guess can be made as to what remedy would be most advisable to recommend in reply. To give definite advice, the insect must first be identified. Applicants are therefore urged to send examples of every pest about which they desire information, and also furnish sample of work when possible.

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Agricultural Experiment Station

OF THE

LOUISIANA STATE UNIVERSITY AND
A. & M. COLLEGE

BATON ROUGE

INFECTED COTTON BOLLS DEVELOPING FROM FLOWERS INOCULATED WITH ANTHRACNOSE DISEASE.

Experiments on Varietal Resistance to the Bean and Cotton Anthracnose Diseases

BY

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Experiments on Varietal Resistance to the Bean and Cotton Anthracnose Diseases.

C. W. EDGERTON AND C. C. MORELAND.

The question of varietal resistance to disease is one that plays an important part in all pathological work. That a disease may attack one variety very severely and be of little importance on a closely related one has been recognized for some time. With some diseases, the selection of varieties or strains that are resistant, forms the chief method of control. Unfortunately our knowledge of varietal resistance or susceptibility to disease is at present deficient. With a few diseases some knowledge has been obtained, but with the great majority we have no definite data. The question is not only of great value from a scientific standpoint but also economically. If we had more knowledge of varietal resistance in different localities, we should be able to increase our crop production to a considerable extent.

From our present knowledge, we know that differences in the host plants do not have the same affect on all diseases. Some diseases confine themselves closely to certain strains or varieties of plants; others apparently affect alike all strains or varieties of a species; while still others are able to attack plants of very diverse relationship. But to what resistance is due, we have but very little knowledge. We merely know that certain varieties show a considerable resistance to a certain disease but we do not know why. Specific knowledge regarding varietal resistance is largely confined to a few groups of diseases, such as the Fusarium wilts, the various rust fungi, etc.

In this bulletin, the results of some experiments on the varietal resistance to two diseases, the bean and cotton anthracnoses, will be given. These diseases are quite similar and are produced by two closely related fungi. The comparison of two closely related fungi in regard to the varietal resistance of the host plants should be of interest.

THE BEAN ANTHRACNOSE.

The bean anthracnose is a disease of beans caused by the fungus, *Colletotrichum lindemuthianum.* The disease does the most damage on the pods where large circular, slimy, reddish, ulcer-like spots are formed, but it also occurs on the leaves and stems. The disease is known to occur on all varieties of wax, green pod, pole, navy, kidney and Lima beans. It has also been reported on the Scarlet Runner. The disease is carried over from fall to spring on the seed and is, on this account, a very difficult one to control by ordinary methods.

The bean plant is an excellent one on which to study varietal resistance to a disease. Bean varieties are well marked and definite. There is practically no crossing of varieties under natural conditions and so there are no intermediate forms between the varieties. It is probable that the presence or absence of intermediate forms plays an important role in the resistance to disease. With well-marked varieties that do not normally hybridize, a disease has an opportunity to become restricted to a single variety; while with plants that do normally hybridize, the presence of all sorts of intermediate forms would tend to prevent the adaptation of a fungus to a single strain.

Previous work on the varietal resistance of beans to the anthracnose has been carried on by Barrus* and reported. He has shown that some strains of the fungus will attack some varieties of beans and not others, although the latter varieties will be attacked by other strains of the fungus.

The experiments described in this paper have been carried on both in the field and in the greenhouse in Louisiana in the years 1914 and 1915. The experiments described by Barrus have been more or less duplicated and results obtained for our conditions. The field experiments were not entirely satisfactory, as neither 1914 or 1915 were very good anthracnose years on account of the dry weather, but results were obtained which give a good check on the greenhouse work.

In the experiments, cultures of *Colletotrichum lindemuthianum* obtained from a number of different sources were used. These came from different parts of the country and also from different varieties of beans. As the disease dies out during the summer months in southern Louisiana, all of the cultures naturally originated in northern sections of the country. Most of them were obtained from beans that were shipped into Louisiana for planting. The cultures used during the course of the work were as follows, the number in each case being the laboratory number of that culture.

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*Barrus, M. F., Variation of varieties of beans in their susceptibility to anthracnose. Phytopathology 1:190-195. 1911.

No. 1651. Culture obtained from bean seed received from Prof. M. F. Barrus of Ithaca, New York, in October, 1913. Variety of bean not noted.

No. 1656. A culture obtained from Messrs. Cook and Martin of New Brunswick, New Jersey, in December, 1913. The fungus had been in culture for a year or more at that time. From what variety the culture was obtained is not known.

No. 1660. A culture obtained from Prof. M. F. Barrus of Ithaca, New York, in January, 1914. From what variety the culture was obtained is not known.

No. 1679. A culture obtained from cotyledons of small bean plants of the variety, Boston Small Pea. Isolated in March, 1914.

No. 1680. A culture obtained from a leaf of a plant of the variety, Boston Small Pea. Isolated in March, 1914.

No. 1681. A culture obtained from cotyledons of plants of the variety, Bountiful. Isolated in March, 1914.

No. 1682. A culture obtained from cotyledons of the variety, Red Kidney. Isolated in March, 1914.

No. 1687. A culture obtained from dried beans of the variety, Davis White Wax. Isolated in April, 1914.

No. 1751. A culture obtained from Boston Small Pea plants that had been inoculated with No. 1679. Reisolated in October, 1914.

No. 1779. A culture obtained from Large White Kidney plants that had been inoculated with No. 1660. Only a very few small spots were present on the plants. Reisolated in October, 1914.

No. 1794. A culture obtained from leaf veins of Large White Kidney plants that had been inoculated with No. 1779 in the greenhouse. A very few spots about a millimeter long were present. Reisolated in April, 1915.

These various cultures were used to inoculate plants of diferent varieties. All of the inoculation work was done by spraying the plants with a suspension of spores from pure culture. No inoculations by puncture were tried, as there is no difficulty in obtaining good infection by spraying the plants with spores.

FIELD EXPERIMENTS.

Field tests were conducted on the spring crop of 1914, the fall crop of 1914 and the spring crop of 1915. Different varieties of beans were grown and at about the time the majority of the varieties had pods about half developed, a number of plants of each variety were sprayed with a suspension of spores of each culture of the fungus. Several sets of inoculations were made during the season so as to obtain some made under the best of weather conditions and also with the pods in the most susceptible stage. All of the inoculated plants, as well as those used for checks, were examined frequently and the relative amount of the disease noted. In the tables that will follow, the amount of the disease is noted by the following symbols: 0, —, +, ++, +++.

FIELD EXPERIMENTS.

0 indicates no infection; —, very slight infection; +, scattering infection; ++, good infection; +++, very good infection.

The infection experiments in the spring of 1914 were much hindered by the weather conditions. The month of May was very dry and the temperature comparatively high, conditions very adverse for a good development of the disease. Most of the inoculations had to be made during this dry weather, and as would be expected, a good infection of the disease was not obtained on all of the susceptible varieties. While an infection in the experiments shows susceptibility to the disease, a non-infection does not necessarily show a resistance. The results of the spring infections of 1914 are shown in Table 1 below. The table shows the relative amount of infection on each variety with each culture of the fungus used.

TABLE 1.

RESULTS OF FIELD INOCULATIONS IN SPRING OF 1914.

VARIETY	1651	1656	1660	1679	1680	1681	1682	1687
Scarlet Runner	0	0	0	0	0	0	0	0
Fordhook's Bush Lima	0	0	0	0	0	0	0	0
Burpee's Bush Lima	0	0	0	0	0	0	0	0
Flageolet Wax	++	+	++	+	+	0	++	+
Bountiful	++	++	+	0	0	+	++	++
Davis White Wax	++	0	0	++	++	++	++	++
Wardwell's Kidney Wax	++	++	+	++	++	++	++	++
Hodson's Long Pod Wax	++	0	+	0	0	0	++	++
Hodson's Long Green Pod	+	0	0	+	0	+	+	++
Longfellow	++	++	++	0	0	++	++	++
Dwarf Horticultural	0	0	0	0	0	+	0	+
Large White Kidney	0	0	0	0	0	0	0	0
Red Kidney	+	0	0	0	0	0	0	++
Boston Small Pea	0	0	0	+	++	0	0	0
White Marrow	+	+	++	0	+	++	++	++

In the fall of 1914, during the month of October, inoculations were again made on a number of bean varieties. The results then obtained are given in Table 2.

TABLE 2.

RESULTS OF FIELD INOCULATIONS IN FALL OF 1914.

VARIETY	1651	1656	1660	1679	1681	1682
Flageolet Wax	+	+	+	0	+++	+++
Bountiful	+++	+++	++	0	++	+++
Davis White Wax	+	+++	+	—	+++	++
Wardwell's Kidney Wax	+	++	+	—	+	+
Hodson's Long Pod Wax	—	—	0	0	—	—
Hodson's Long Green Pod	0	0	0	0	—	0
Longfellow	+	++	+++	0	++	+++
Dwarf Horticultural	—	—	—	0	+	—
Large White Kidney	0	0	—	0	0	0
Red Kidney	—	+	+	0	+++	++
Boston Small Pea	0	0	0	+	0	0
White Marrow	+	+	—	0	—	—

In the spring of 1915, different bean varieties were again inoculated in the field with four of the anthracnose cultures. These were cultures which had previously shown variations in regard to their ability to infect different varieties. The results of this experiment are given in Table 3 below.

Table 3.

RESULTS OF FIELD INOCULATIONS IN SPRING OF 1915.

Variety	1681	1682	1751	1794
Flageolet Wax	—	—	—	—
Bountiful	++	++	—	+
Davis White Wax	++	+++	++	++
Hodson's Long Pod Wax	++	0	0	0
Longfellow	+	+	—	—
Dwarf Horticultural	+	—	—	—
Large White Kidney	0	0	0	0
Red Kidney	++	++	0	+
Boston Small Pea	0	0	++	0
White Marrow	+++	++	—	+

GREENHOUSE INOCULATIONS.

Greenhouse experiments were also conducted during the winter of 1914-15. The young plants of the different varieties were grown in pots until they were four or five inches high and then used for inoculation purposes. In this work only one culture was used at a time. All of the different varieties were inoculated with the culture and the plants kept until sufficient time had elapsed to obtain a good infection. The plants were then removed and the test duplicated with another culture of the fungus. The plants were covered with bell jars lined with moist paper for two days after the inoculation to give a perfect chance for infection. There was very little chance in these experiments for a plant to remain healthy unless it was resistant to the culture used. The spots would usually begin to show within four to six days after the inculation and would reach their full development in about two weeks. The results of the greenhouse inoculations are given in Table 4 below.

Table 4.

RESULTS OF GREENHOUSE INOCULATIONS.

Variety	1651	1656	1660	1679	1681	1682	1751	1779
Scarlet Runner	0		0	0	0	0	0	0
Fordhook's Bush Lima				++				++
Flageolet Wax				++	+++	+++		
Bountiful	+++	+++	++	+++	+++	+++	++	+++
Davis White Wax				++	+++			
Wardwell's Kidney Wax	+++	+	+++	++	+++	++	++	+++
Hodson's Long Pod Wax	++	+++	++	++	+++	++	++	+++
Hodson's Long Green Pod	++	—	—	++	+++		++	+++
Longfellow	+++	+++	+++	++	+++	++	++	+++
Dwarf Horticultural	++	—	+++	++	+++	+++	++	+++
†Large White Kidney	0	—	0	—	—	0	0	—
Red Kidney	+++	++	+++	0	+++	+++	—	+++
Boston Small Pea	+	—	++	+++	+++	—	+++	++
White Marrow		—	0	+++	+	++	0	+

† The large white kidney plants that showed infection were only very slightly affected. There were only two or three very small spots about a millimeter in length on the under side of the leaf veins. These spots did not increase in size and would not have been noticed if a careful examination of the plants had not been made.

RESULTS OF THE INOCULATIONS.

An examination of the above tables will show that practically the same results were obtained as were obtained by Barrus in his experiments. Cultures of the bean anthracnose show variations in regard to their virulence on different varieties of beans. Briefly, it may be said that, while each variety of bean does not have its own strain of the fungus, there are certain varieties of beans that are attacked much more severely by certain strains of the fungus than by others and some varieties are practically immune to certain strains. The points which seem of particular importance may be briely mentioned.

1. Beans of the snap varieties such as Wardwell's Kidney Wax, Bountiful, Davis White Wax, Flageolet Wax and Longfellow seemed to be susceptible to all of the strains of the fungus that were used in the experiments. In some instances there was a slight difference in the relative amount of infection but this was usually not very marked. It is possible that other strains of the

fungus may be found that would not attack these varieties, but it is of importance to note that strains which showed considerable differences in regard to their ability to attack other varieties seemed to attack all of these snap varieties.

2. There was no culture among the lot that would develop satisfactorily on the Scarlet Runner or the Large White Kidney. It is known, however, that these beans are not immune to the anthracnose. Barrus has had strains that would attack these varieties and Massee* has also reported the disease on the Scarlet Runner in Europe. A few times during the course of this work, a limited number of very small spots developed on the Large White Kidney plants, but these always remained small and did not seem to penetrate to any distance in the host tissue. These spots, however, generally produced a limited amount of conidia and several times the fungus was reisolated from them. The immunity did not seem to be due to a lack of infection but rather to the inability of the fungus to grow in the tissues of the plant.

3. Some of the varieties of the beans used, especially the Boston Small Pea and the Red Kidney, showed considerable resistance to certain strains of the fungus but were very susceptible to other strains. The Boston Small Pea was injured very badly by a culture obtained originally from this variety while the infection produced by other strains was not usually very marked. It is important to note, however, that these other strains were able to produce some infection on the Boston Small Pea bean. The Red Kidney bean seemed to be attacked by all of the strains with the exception of the ones that originally came from the Boston Small Pea. It should be stated, however, that the strain that came originally from the Red Kidney bean caused a somewhat greater infection on this variety than the other strains.

There seemed to be three distinct types among the different cultures studied. One of them, perhaps best represented by No. 1681, was able to infect all of the varieties of the species, *Phaseolus vulgaris,* that were tried with the exception of the Large White Kidney. Some of the varieties, especially the Boston Small Pea, did not show as heavy an infection as some of the others, but the infection in some cases could not be classed other than good. In figure 1 are shown plants affected with this fungus: a, Boston Small Pea; b, Bountiful; c, Large White Kidney; d, Red Kidney. The Large White Kidney shows no infection, the Boston Small Pea shows considerable infection but not enough to kill all of the plants, while the Bountiful and Red Kidney are entirely dead.

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*Masse, G., French bean canker. Gard. Chron. 3 ser., Vol. 23, No. 594: 294. 1898.

A second type, best represented by No. 1679, caused a severe infection on the Boston Small Pea, fair to good infection on the snap varieties, but practically no infection on the Large White Kidney or the Red Kidney. In figure 2 are shown plants affected with this fungus: a, Boston Small Pea; b, Bountiful; c, Large White Kidney; d, Red Kidney. The Boston Small Pea plants are badly infected, the Bountiful slightly less, while the Large White Kidney and the Red Kidney show no infection.

The third type, best represented by No. 1682, shows a very heavy infection on the Red Kidney, somewhat less infection on the snap varieties, a considerable less infection on the Boston Small Pea, and no infection on the Large White Kidney. In figure 3 are shown plants affected with this fungus: a, Boston Small Pea; b, Bountiful; c, Large White Kidney; d, Red Kidney. The Red Kidney plants are very badly affected, the Bountiful somewhat less, the Boston Small Pea still less, and the Large White Kidney no infection.

There may be many other strains of the bean anthracnose and undoubtedly there are some others. There are, without doubt, strains which will attack the Large White Kidney and the Scarlet Runner. Possibly also strains may be found that will attack cowpeas and other legumes. Chester* has reported an anthracnose on cowpea seed and it would not be surprising if this were really a strain of the bean anthracnose. And perhaps some of the other described species of Gloeosporium and Colletotrichum on legumes will be found to be but strains of *Colletotrichum lindemuthianum* and it is possible that we may find bridging species of host plants that will connect these forms absolutely.

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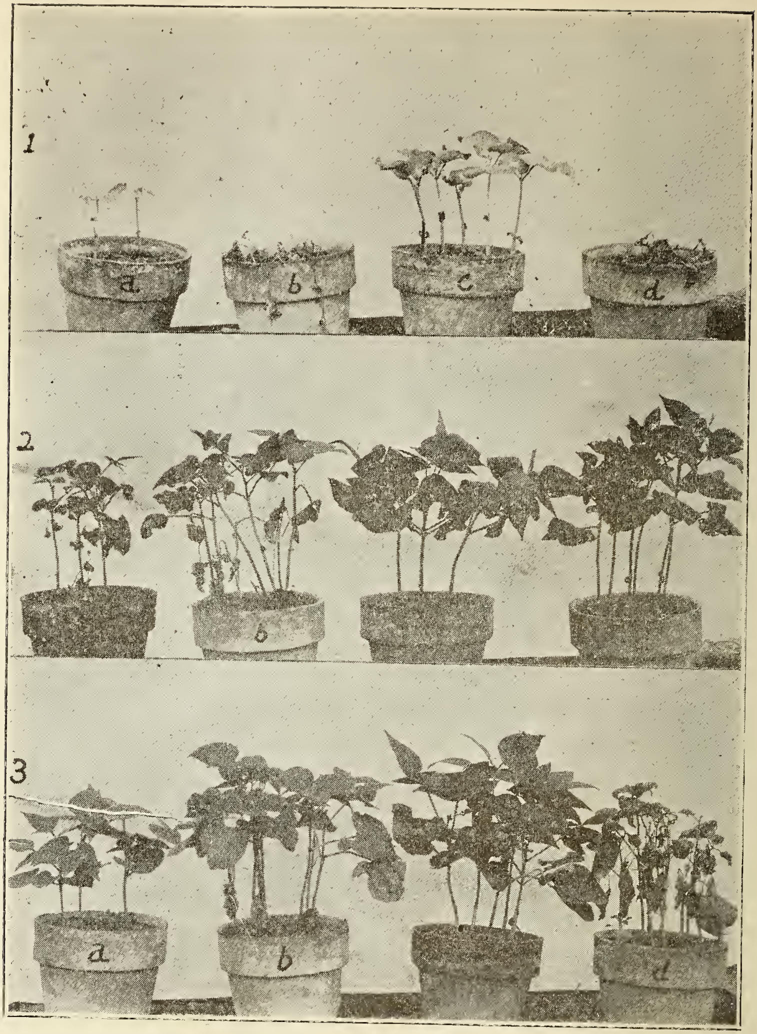
*Chester, F. D., Diseased cowpea seed. Del. Agr. Exp. Station, 6th Annual Report, 1893:110-111. 1894.

An interesting point in these experiments was the successful infection of some of the cultures on Fordhook's Bush Lima bean, a variety of the species *Phaseolus lunatus.* Here we find cultures that will attack varieties of different species and yet will not infect all varieties of the same species.

The origin of these different fungous strains is not perfectly clear. There seems to be two possibilities. (1) The original bean fungus may have been able to infect all or at least a great many varieties of beans and their relatives. Later particular strains, perhaps isolated in certain sections of the world, might have lost their power to infect certain varieties that were not present in that locality. (2) Or it is possible that the original fungus was confined to one or at least a few varieties such as the soft podded snap varieties. As time passed, certain strains gradually adapted themselves to other types of beans. The latter possibility seems more probable. It is not unlikely that the fungus which originally occurred on a wax variety gradually adapted itself to a kidney variety and after some time developed even better on this than on its original host. Another strain in the same way would adapt itself to the Navy type of beans. The bean anthracnose is not very variable and would not readily change after becoming adapted to a special host.

An attempt was made to adapt a strain of the bean anthracnose to a bean variety that was not susceptible. As has been noted, under the most favorable circumstances culture No. 1660 would cause a very slight infection on the Large White Kidney bean. It seemed possible to obtain a strain that would cause a good infection on the Large White Kidney by repeated reisolations from this variety. There was difficulty in culturing the fungus from the very small spots but after some attempts it was carried through three generations on the Large White Kidney bean. Cultures No. 1779 and 1794 were ones obtained in this manner. As far as this work went, absolutely no change was noticeable in the fungus. At the end, it would not infect the Large White Kidney a bit better than the original culture. Of course, if this work had been carried on through several years, the fungus might have gradually adapted itself to this variety.



Effect of different cultures of *Colletotrichum lindemuthianum* on bean varieties. The variety in each case is as follows: *a*, Boston Small Pea; *b*, Bountiful; *c*, Large White Kidney; *d*, Red Kidney. Fig. 1. Plants inoculated with culture 1681 obtained from Bountiful beans. Fig. 2. Plants inoculated with culture 1679 obtained from Boston Small Pea beans. Fig. 3. Plants inoculated with culture 1682 obtained from Red Kidney beans.

THE COTTON ANTHRACNOSE.

The cotton anthracnose is a disease of cotton caused by the fungus, *Glomerella gossypii (Colletotrichum gossypii)*. This disease causes the most injury to cotton bolls but it also occurs on the leaves and stems of the plant. The disease shows on the bolls in the form of spots or by a general rot. As the disease is carried over from fall to spring on the seed in the same manner as the bean anthracnose, it is also difficult to control. All varieties of cotton seem to be more or less affected.

Cotton is not represented by well-marked varieties as the bean plant is. Cotton varieties cross very readily in the field and all intergrading forms between the varieties can be found. The lack of distinct types and the presence of intergrading forms hinders materially the specialization of fungous strains to particular types.

There is very little reliable data published on the varietal resistance of cotton to the anthracnose disease. Many counts have been made at various times in variety plots but these do not answer the requirements because the various factors in the spread of the disease have not been taken into account. A resume of these previous counts has been made in a previous publication* and it is not necessary to include it here. It should be said, however, that these counts have given extremely variable results and do not give much authentic information regarding varietal resistance. In order to get reliable data, the plants must have an equal chance to become infected and this can only be obtained by artificial means

Data compiled for this bulletin were obtained by two methods, by natural infection and by artificial infection. All of the work has been conducted in the field as it is impossible to grow any number of cotton plants to maturity in a limited sized greenhouse. In the natural infection work the varieties were grown between rows of badly infected plants and the percentage of infection obtained. In the artificial infection work the flowers were inoculated with pure cultures of the anthracnose fungus.

The natural infection work has been carried on by several different southern Experiment Stations. The project was initiated by Professor H. R. Fulton of the North Carolina Experiment Station. Seed of different varieties was sent to workers at the different stations and this was used in the work. The seed used was supposed to be free of the disease but this was not always the case. It is a very difficult matter to obtain seed that is known to be absolutely free of the disease. Growers in each case claimed green house. A very few spots about a millimeter long were the seed to be healthy and they were without doubt honest in their convictions but a limited amount of disease in a field is very liable to be overlooked. This seed was planted in the field so that each variety was adjacent to a row planted from badly infected seed. The object of the experiment was to see what percentage of the bolls would become infected from the adjoining rows.

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*Edgerton, C. W., The rots of the cotton boll. Louisiana Agr. Exp. Station Bulletin 137:49-51. 1912.

This work was begun in 1914 when ten different varieties were used. Before planting, samples of all of the seeds were tested for the presence of anthracnose spores. The estimated number of anthracnose spores on three hundred seeds of each variety is given in Table 5.

TABLE 5.

NUMBER OF ANTHRACNOSE SPORES ON COTTON SEED TESTED.

VARIETY	No. of spores on 300 seeds
Modella	0
Cook's Improved	0
Sunbeam	0
Lewis Hybrid No. 63	0
Dix-afifi	0
Half and Half (North Carolina)	2,500,000
Half and Half (Georgia)	165,000
Mebane Triumph	63,000,000
Covington-Toole	165,000
Simpkins	6,325,000

The above table shows that some of this seed was very heavily infected. In fact, the Mebane Triumph seed had the heaviest infection of any seed that has ever been tested at the Experiment Station laboratory. The infection was so bad that many of the seeds were pink from the abundance of spores.

Seed that had been gathered in infected fields was also received to be planted alongside of the varieties. Two lots of this infected seed was obtained, one from South Carolina and the other from North Carolina. These two lots will be designated in this bulletin as *South Carolina Infection Seed* and *North Carolina Infection Seed.* These were also tested in the laboratory and it was found that the South Carolina seed had 700,000 spores to 300 seeds while the North Carolina seed had 1,760,000 to the same number.

NATURAL INFECTION EXPERIMENTS.

The seed was all planted so that a row of each variety had an infection row on each side of it. This gave a chance for the spread of the disease to all plants. The season of 1914 was an excellent one for the development of the anthracnose disease in Louisiana. There was a great deal of wet weather and the boll weevil infestation in the experimental plots was very heavy, making conditions very favorable for good infection. This experiment was conducted in duplicate at Baton Rouge and was also tried in North Carolina by Professor Fulton and in Georgia by Professors Lewis and Rast. The percentage of infection obtained in the two test plots at Baton Rouge and by the other workers in North Carolina and Georgia is given in Table 6. The percentage of infection at Baton Rouge was determined by obtaining the actual percentage of infected bolls on twenty-five hills. The methods employed at the other stations were not noted in the reports.

TABLE 6.

NATURAL INFECTION TESTS IN 1914; PERCENTAGE OF INFECTION.

VARIETY	LOUISIANA			GEORGIA		NORTH CAROLINA,
	First Plot	Second Plot	Average	Rast	Lewis	Fulton
Lewis Hybrid No. 63	....	7	7	15	....	6
Half and Half (N. C.)	8	12	10	11	14	5
Cook's Improved	9	13	11	5	17	19
Simpkins	13	10	12	14	12	5
Dix-afifi	13	11	12	5	....	6
Sunbeam	10	15	13	6	15	8
Half and Half (Ga.)	22	8	15	7	42	20
Covington-Toole	15	15	15	13	....	6
Modella	14	15	15	4	13	7
Mebane Triumph	15	17	16	17	....	15
S. C. Infection Seed	7	9	8	17	....	21
N. C. Infection Seed	14	11	13	....	43	14

The above table shows a great variation in regard to the relative infection of the different varieties in the different localities and even in the different tests at Baton Rouge. However, the experiment does not give any evidence that any one variety is markedly more resistant than any other variety.

In 1915, this experiment was again conducted at Baton Rouge, seed being furnished by Professor Fulton as in the previous year. The infection on the seed used was found to be as follows:

TABLE 7.

NUMBER OF ANTHRACNOSE SPORES ON COTTON SEED IN 1915.

VARIETY	No. of spores on 300 seed
Sunbeam	0
Half and Half	0
Simpkins	1,650,000
Covington-Toole	250,000
Dix-afifi	100,000
Modella	250,000
Willet's Red Leaf	1,000,000
Infection Seed	1,650,000

The experiment was carried on in the same way as in the preceding year except that it was not conducted in duplicate. Also the field was located in a place where the boll weevil infestation was very light, this tending to decrease the amount of anthracnose infection. The results of this experiment are given in Table 8 below. In this table the varieties and infection rows are listed just as they occurred in the field, thus giving the percentage of infected bolls on each variety as compared to the adjacent infection rows.

TABLE 8.

NATURAL INFECTION TEST IN 1915; PERCENTAGE OF INFECTION.

Row 1.	Infection row	12.3
Row 2.	Simpkins	6.7
Row 3.	Infection row	4.5
Row 4.	Covington-Toole	5.0
Row 5.	Infection row	3.4
Row 6.	Sunbeam	2.4
Row 7.	Infection row	3.9
Row 8.	Half and Half	8.2
Row 9.	Infection row	3.4
Row 10.	Modella	2.6
Row 11.	Infection row	3.3
Row 12.	Willet's Red Leaf	2.1
Row 13.	Infection row	2.6
Row 14.	Dix-afifi	1.5
Row 15.	Infection row	7.1

This table also shows a lack of any great variation among the varieties in regard to their susceptibility or resistance to the anthracnose disease. There is no more variation among the varieties than among the different infection rows that were planted from the same seed.

ARTIFICIAL INFECTION EXPERIMENTS.

During the seasons of 1913, 1914 and 1915 artificial inoculation experiments were also conducted. Young bolls will readily take the disease if spores and a sufficient amount of moisture are present. By experience it has been found that the most satisfactory artificial infection is obtained by inoculating the flowers. A small amount of a suspension of spores in water is placed in the open flower late in the afternoon. The flower soon after closes and dies and the spores are held in an excellent condition for germination. The germinating spores or mycelium comes in contact with the very young developing bolls underneath and cause infection. This infection may occur at almost any place on the boll but is more frequent at the tip. The fungus is able to grow down through the old dead pistil into the boll. Sometimes the whole center of the boll decays before the capsular tissue is destroyed but more often the rot spreads downwards through both the center of the boll and the capsular tissue. The figure on the front page of this bulletin shows bolls that have become infected following flower inoculation. In all of the artificial infection work discussed in this bulletin, the inoculated flowers were marked by tags and all of the bolls were examined from two to three weeks after the inoculation.

The first test was made in the summer of 1913 when flowers of four different varieties were inoculated. The results of this test are given in Table 9. A great many more inoculations were made than are shown but they were made at a time when shedding was very severe and only a few bolls were left.

Table 9.

RESULTS OF FLOWER INOCULATIONS IN 1913.

Variety	No. of bolls	No. diseased	% diseased	% diseased at tip
Toole	26	20	77	35
Cook's Improved	64	46	72	14
Simpkins	88	37	42	16
Cleveland Big Boll	29	14	48	14

In 1914, a greater number of varieties were used and a much larger number of inoculations. Two sets of experiments were conducted during the season. For the first set, inoculations were made daily from June 23 to July 2, and for the second set from July 6 to July 24. A period of very wet weather occurred between July 2 and July 6, causing a very heavy natural infection, and it seemed best to consider the two sets separately. Flowers treated with pure water were used as checks. In Table 10 is given the results of the set of inoculations made between June 23 and July 2. For each variety there is listed the number of bolls that developed from the inoculated flowers, the percentage of infected bolls and the percentage of bolls infected at the tip.

TABLE 10.

RESULTS OF FLOWER INOCULATIONS FROM JUNE 23 TO JULY 2, 1914.

VARIETY	CHECKS			INOCULATED		
	Bolls	% Infected	% Infected at tip	Bolls	% Infected	% Infected at tip
Cook's Improved	29	34.5	7.0	30	76.7	30.0
Modella	45	75.6	0.0	41	78.1	19.5
Sunbeam	33	48.5	3.0	37	75.7	27.0
Simpkins	46	50.0	4.3	61	82.0	24.6
Piedmont Early	42	66.7	14.3	53	88.7	30.2
Half and Half (Ga.)	33	33.3	3.0	20	65.0	30.0
Covington-Toole	28	39.3	3.6	34	79.4	38.2
Trice	45	51.1	4.4	57	82.5	38.6
Perry's Improved	60	58.3	5.0	83	88.0	33.8
Half and Half (N. C.)	62	45.1	9.7	50	80.0	40.0
Mebane Triumph	26	57.7	4.0	40	90.0	42.5
Lewis Hybrid No. 63	36	16.7	5.6	52	73.1	38.4
Dix-afifi	69	50.7	2.9	54	79.6	42.6
Rublee's Defoliate	25	48.0	4.0	39	95.0	38.4
Wannamaker's Pedigree Cleveland Big Boll	91	68.1	3.3	83	91.6	43.4

The results of the second set of experiments from July 6 to July 24 are given in Table 11.

TABLE 11.

RESULT OF FLOWER INOCULATIONS FROM JULY 6 TO JULY 24, 1914.

VARIETY	CHECKS			INOCULATED		
	Bolls	% Infected	% Infected at tip	Bolls	% Infected	% Infected at tip
Cook's Improved	33	6.0	0.0	29	34.5	10.4
Modella	45	11.1	0.0	77	47.3	25.9
Sunbeam	35	11.4	2.9	27	37.0	26.0
Simpkins	39	10.3	0.0	40	55.0	30.0
Piedmont Early	17	23.5	5.9	23	56.5	30.4
Half and Half (Ga.)	36	8.3	0.0	49	42.8	30.6
Covington-Toole	42	7.1	0.0	44	43.2	27.3
Trice	22	9.1	4.5	27	48.2	25.9
Perry's Improved	35	14.3	2.8	22	59.1	31.8
Half and Half (N. C.)	44	4.6	0.0	44	43.2	34.1
Mebane Triumph	33	15.1	9.1	26	46.3	34.6
Lewis Hybrid No. 63	80	1.3	0.0	69	49.3	40.6
Dix-afifi	35	8.6	0.0	22	63.6	36.2
Rublee's Defoliate	25	16.0	8.0	17	64.7	47.1
Wannamaker's Pedigree Cleveland Big Boll	87	9.2	0.0	74	56.7	43.2

Regarding the total infection, Table 10 should not be considered in discussing the results of the above experiments. The weather conditions caused such a heavy infection even on the checks that the results are of but little value. The infection percentage in Table 11, however, is more reliable. The infection on the check bolls is not very heavy and the increase on the inoculated bolls gives us an idea in regard to the susceptibility of the different varieties. But in discussing the results, the question of probable error must be taken into consideration. The uninoculated check bolls show a variation from 1.3% to 23.5% and as wide a variation must be allowed with the inoculated bolls. The percentage of infection on the inoculated bolls varied between 34.5% and 64.7%. Allowing for a possible error as large as on the checks, we can only draw the conclusion that the experiments have not shown any marked differences in regard to the susceptibility to the anthracnose disease.

Regarding the percentage of infection at the tips of the bolls, it would seem that both sets of inoculations could be considered. There is not a high percentage of infection at the tips of the check bolls of either set. Consequently in Table 12, the results of both sets in regard to tip infection are combined.

TABLE 12.

TIP INFECTION IN INOCULATION EXPERIMENTS IN 1914.

VARIETY	CHECKS		INOCULATIONS	
	Bolls	% infected at tip	Bolls	% infected at tip
Cook's Improved	62	3.2	59	20.3
Modella	90	0.0	118	23.7
Sunbeam	68	2.9	64	26.6
Simpkins	85	2.3	101	26.7
Piedmont Early	59	11.8	76	30.3
Half and Half (Ga.)	69	1.5	69	30.4
Covington-Toole	70	1.4	78	32.1
Trice	67	4.5	84	33.2
Perry's Improved	95	4.2	105	33.3
Half and Half (N. C.)	108	5.6	94	37.2
Mebane Triumph	59	6.8	66	39.4
Lewis Hybrid No. 63	116	1.7	121	39.7
Dix-afifi	104	2.0	76	40.8
Rublee's Defoliate	50	6.0	56	41.1
Wannamaker's Pedigree Cleveland Big Boll	178	1.7	157	43.3

The percentage of infection at the tips of the inoculated bolls shows a variation from 20.3% to 43.3%. The check bolls show an infection as high as 11.8% in one instance but in no other case higher than 7%. This shows that the error in the inoculation results due to natural infection is probably not above 7%, though a possible greater error may be present. Taking account of the possible error due to natural infection, there is still some variation among the different varieties. Yet the differences are not marked enough to show any remarkable variation in regard to the resistance to the anthracnose disease.

In the summer of 1915, another set of inoculation experiments was conducted. Flowers of five different varieties were inoculated. A greater number of flowers were inoculated than in the previous years, thus decreasing the error. The natural infection in the field was not heavy, running from 2% to 5%, so that most of the infection obtained in the tests came from the inoculations. Data on tip infection was not obtained, only the total infection being considered. The results of this experiment are given in Table 13.

TABLE 13.

FLOWER INOCULATION EXPERIMENTS IN 1915.

VARIETY	Bolls	No. infected	% infected
Simpkins	498	132	26.5
Modella........................	387	129	33.3
Cook's Improved..................	127	47	36.4
Sunbeam	345	138	40.0
Covington-Toole	408	167	40.9

An examination of this table shows a slight variation among the varieties in regard to the amount of infection. Simpkins shows less infection than some of the other varieties, yet the infection obtained with this variety shows that it is quite susceptible.

Considering all of the experiments with the cotton anthracnose that have been described, it can only be said that the different varieties do not show any very marked difference in regard to the susceptibility to anthracnose. It is probably a fact that there is less actual loss in a field with some of the small bolled cottons such as Simpkins but this is not due so much to the greater resistance of the variety but rather to the fact that the bolls are smaller, more numerous and develop quicker, and also are not shaded so much on account of the smaller size of the plants. Cook's cotton has a reputation in some parts of the cotton belt of being one of the most susceptible varieties, and while the experiments with artificial infection described in this bulletin have shown that it is susceptible, they do not show any greater susceptibility than with many other varieties.

It is believed by the writers that the great difference in infection on different varieties that is often noticed in the field is not due so much to the difference in susceptibility of the varieties but rather to the infection that was on the seed when planted. If seed of one variety comes from a badly infected field it is only reasonable to suppose that the plants growing from that seed will show a high infection, but if the seed is free of infection, the chance of a heavy outbreak of the disease is slight. This phase of the problem, however, has already been discussed in a previous bulletin.

VIRULENCE OF DIFFERENT CULTURES.

No mention has been made in the above discussion regarding the cultures that were used and their virulence. As the bean anthracnose cultures showed a remarkable difference in regard to their ability to infect different varieties, the question might be asked whether the cotton cultures might not show a similar adaptation. From the evidence at hand, however, this does not seem to be the case. During the course of this work during several years, a large number of different cultures have been used. These have been used on a large number of varieties of cotton and no variety has shown any marked resistance to any one of them. To be sure, cultures do not all show the same virulence; some cultures are comparatively weak* while others are very virulent, but no culture that has been used has shown any marked virulence on one variety and weak virulence on another. In the experiments which have been described on the previous pages, many different varieties are seen to be badly attacked by the same culture. This either shows that there is no marked specialization with the cotton anthracnose, or else the cultures that were used had not become specialized. While there is no evidence that there is any specialization, it is barely possible that strains may be found in isolated places which have become adapted to particular varieties of cotton and are more virulent on these. Considering, however, that cotton crosses readily and does not have the well-marked varieties as is the case with beans, this seems unlikely to the writers.

In the experiments in 1914, two different cultures were used in the inoculation work. In the tables on the preceding pages these results were united, but it may be of interest to give the percentage of tip infection with the two cultures. One culture, numbered 1677, was obtained from infected seed in Louisiana, though the variety was not noted at the time. The other culture, numbered 1694, was obtained from infected Triumph seed from North Carolina, the same seed described in a previous page as being so badly infected. The percentage of tip infection on the different varieties with these two cultures is given in the following table.

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*Barre (South Carolina Agr. Exp. Sta. 26th Annual Report, 1912-13 :16-17. 1913) has reported a culture obtained from dead stalks that would not produce any infection on living bolls, but it is questionable whether he really was working with a culture of *Glomerella gossypii*. A closely related fungus, *Glomerella cingulata*, is found very abundantly on all kinds of dead plants in the southern states and it is possible that his culture from a dead cotton stalk was of this species.

TABLE 14.

TIP INFECTION WITH DIFFERENT ANTHRACNOSE CULTURES.

VARIETY	CULTURE No. 1677		CULTURE No. 1694	
	No. bolls	% Infected at tip	No. Bolls	% Infected at tip
Cook's Improved	24	20.8	35	20.0
Modella	54	20.4	64	26.6
Sunbeam	29	31.0	35	22.9
Simpkins	50	32.0	51	21.6
Piedmont Early	34	29.4	42	30.9
Half and Half (Ga.)	37	24.3	32	37.5
Covington-Toole	38	26.3	40	37.5
Trice	46	34.8	38	34.2
Perry's Improved	44	36.7	61	31.1
Half and Half (N. C.)	55	30.9	39	46.1
Mebane Triumph	34	44.1	32	34.4
Lewis Hybrid No. 63	64	41.0	57	28.6
Dix-afifi	44	31.8	32	53.1
Rublee's Defoliate	27	44.4	29	37.9
Wannamaker's Pedigree Cleveland Big Boll	76	43.4	81	43.2

An examination of the above table shows that both cultures gave good infection on all of the cotton varieties used and in only a few cases was the variation very marked. Considering the small number of bolls of each variety used, a considerable variation in infection could be expected. Checks were used at the same time and these showed an infection varying from 0% to 12.8%. An examination of the table shows that only the Half and Half cottons and the Dix-afifi differ in the amount of infection more than 12.8%.

It is interesting to note in Table 14 that the Mebane Triumph cotton showed a higher infection with culture No. 1677 than it did with the one direct from Triumph seed, No. 1694. If there had been any specialization, the opposite would have been expected.

The only conclusion that can be drawn from the data obtained is that no evidence has been obtained which would show that there is any marked specialization with cotton anthracnose strains. In the opinion of the writers there seems to be two reasons for this lack of specialization. (1) The cotton anthracnose fungus, like the closely related species on the apple, *Glomerella cingulata,* seems to be quite variable and is able to adjust itself to variations in the host plant. The cotton anthracnose fungus will even develop to some extent on species of plants other than cotton. This is in marked contrast to the bean anthracnose, which does not show any marked variability or the ability to adjust itself to slight variations in the host. (2) On account of the ease with which cotton crosses, well-fixed and well-marked varieties do not occur. While there are marked differences between typical plants of different varieties, it is usually possible to find all intergrading forms between them. These intergrading forms hinder the development of resistant varieties as they form a bridge which allows the fungus to pass from one variety to another. These forms also hinder the development of distinct fungus strains as is found in the bean anthracnose.

CONCLUSIONS.

The conclusions which may be drawn from the experiments described on the previous pages may be briefly enumerated.

(1) The bean anthracnose fungus, *Colletotrichum lindemuthianum*, is composed of a number of different strains, these affecting bean varieties differently. Some beans, like the snap varieties, seem to be susceptible to many different strains of the fungus, while other varieties are fairly resistant to some strains but very susceptible to others. Some bean varieties are practically immune to some strains of the fungus.

(2) The existence of these different fungus strains seems to be due to two things. The bean anthracnose fungus is not very variable and so is unable to adjust itself to slight variations in the host plant. Furthermore, bean varieties are well marked and definite. The varieties do not cross readily and there are no intergrading forms between them. The lack of intergrading forms permits the specialization that is seen in the different strains.

(3) The cotton anthracnose fungus, *Glomerella gossupii*, does not seem to be composed of different strains, as is the bean anthracnose fungus, as all of the cultures which were used in the experiments were able to infect a large number of cotton varieties.

(4) The cotton anthracnose fungus is more variable than the bean anthracnose fungus and is more able to adjust itself to variations in the host plants. Furthermore, cotton varieties cross very readily and the intergrading forms which result hinder the development of the special strains.

(5) Cotton varieties do not show any marked variability in regard to their resistance to the anthracnose disease. All of the varieties that have been tried seem to be very susceptible, and while the different varieties have in some cases given slightly different results, these have not been marked enough to be considered important.

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OF THE

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AND A. & M. COLLEGE

BATON ROUGE

The Direct Determination of Sucrose in the Presence of Reducing Sugars

BY

M. A. SCHNELLER

THE DIRECT DETERMINATION OF SUCROSE IN THE PRESENCE OF REDUCING SUGARS

The methods proposed for the direct determination of sucrose in the presence of reducing sugars have not proved quite successful and none has come into general use. They are based on the destruction of the rotary power of reducing sugar by the action of alkali, effecting a rearrangement of the glucose and fructose molecules and their transformation into an inactive mixture of glucose, fructose and mannose. The various analytical methods are reviewed in Browne's Handbook of Sugar Analysis, p. 302, also in Louisiana Bulletin No. 135, p. 47, where a modification of the method of Pellet and Lameland, employing hydrogen peroxide in addition to alkali is described by Cross and Taggart. They found Pellet's method unreliable, but although their own modification gave results in close agreement with Clerget's inversion method for sugar house products, it yielded too low figures with pure sucrose. This fact, illustrated in Tables I and II, might be

TABLE I

25 cc of a sucrose solution mixed with varying amounts of reducing sugar (glucose-fructose) were heated with 2 cc NaOH (40° Be) and .45 cc H_2O_2 (3%) at 55°C for 20 minutes, on cooling neutralized and made up to 100 cc.

g Reducing Sugar	Polarization V
0.	24.25
0.25	24.45
0.5	24.85
1.0	25.05
1.5	25.05
2.0	25.05
0. Without NaOH, H_2O_2	24.7

TABLE II

Solution A containing 5% fructose
" B " 12.5g invert sugar syrup (*) 77% (with 3% sucrose) in 200 cc
" C " 25% sucrose

were mixed in varying proportions and heated with 2 cc NaOH (40° Be) and 45 cc H_2O_2 (3%) in boiling water bath for 20 minutes, on cooling neutralized and made up to 100 cc.

Mixture	Polarization °V
25 cc A	0.
50 cc A	0.
25 cc B	0.2
50 cc B	0.38
25 cc C	22.1
25 cc A + 25 cc C	23.4
25 cc B + 25 cc C	23.4
25 cc C without H_2O_2	24.6
25 cc C without NaOH, H_2O_2	24.8

* An invert sugar syrup prepared by inversion of 1000g sucrose +250g water with ½ cc HCl at 95-97° (Z. Zuckerind. Böhmen, 1915, p. 282); it contained 2.9 per cent sucrose according to Clerget's method.

explained by a slight destruction of sucrose by hydrogen peroxide in alkaline solution analagous to the action of Fehling solution on sucrose in the presence of reducing sugars. It was therefore sought to avoid the use of an oxidant for the decoloration of the dark reaction products formed on heating reducing sugars with alkali, and sulphur dioxide and sodium hydrosulfite were substituted for this purpose. The detail of the method employed is as follows:

The normal weight of material (= 26g.) is dissolved in a 200 cc flask, the solution clarified with lead subacetate, filled up to the mark and filtered. The excess of lead is removed from the filtrate by gaseous SO_2 or dry K_2HPO_4.

A 50 cc measuring flask is filled up to the mark with the filtrate and 1 cc of KOH (36°Be.) added. After heating in a boiling water bath for about 45 minutes, a few crystals of Blankit are added to the hot solution, which after cooling is neutralized with SO_2 gas. As soon as the liquid becomes acid, the color brightens up very considerably. There is no danger from inversion by this way of procedure, as is shown in Tables XI and XIV. The flask is filled up to the 55 cc mark and the solution polarized. The following table gives a comparison of results by Clerget-Herzfeld's inversion method and the one just described:

TABLE III

Product	% Sucrose by Clerget	% Sucrose by above method
Blackstrap	33.1	31.24
Blackstrap	33.0	32.12
Blackstrap	34.0	32.46
Blackstrap	33.8	32.12
1st. Molasses	46.5	45.22
1st. Molasses	47.5	46.64
1st. Molasses	47.3	46.74
Raw Sugar	91.9	92.8
Raw Sugar	96.85	96.8
RawS ugar	91.4	91.1

The results agree fairly closely for the raw sugars, but regular differences of 1 to 2 per cent occur with molasses. These must be ascribed to a residual levorotation of reducing sugar. This explanation becomes still more apparent from the following analysis of artificial mixtures of sucrose and reducing sugars:

TABLE IV

Solution A containing 200g sucrose (= 76.78% of normal weight) in 500 cc
" B " 62,5g invert sugar syrup of 77% (+2.9% sucrose) in 500 cc

were mixed in varying proportions and the volumes completed to 200 cc:

Mixture	% Invert Sugar	% Sucrose		
		Calculated	By inversion	By above method
50 cc A		76.78	76.7	
100 cc B			1.4	*
50 cc A+100 cc B	5	78.18	78.06	76.12
50 cc A+80 cc B	4	77.9	77.9	76.12
50 cc A+60 cc B	3	77.62		76.12
50 cc A+40 cc B	2	77.34	77.2	76.12
50 cc A+20 cc B	1	77.06	77.3	76.34

* Levorotary (compensating 1.32% sucrose.)

The results obtained by the direct method show differences from the actual sucrose contents varying from 0.7 per cent for a concentration of 1 per cent invert sugar to 2 per cent for a concentration of 5 per cent invert sugar. We now proceeded to ascertain by what change of the working conditions a more complete reduction of the invert sugar rotation could be accomplished. 50 cc of a solution of the above mentioned invert sugar were heated for 45 minutes with varying amounts of alkali as follows, a trace of hydrosulfite added, after cooling neutralized with SO_2 and made up to 50 cc.

TABLE V

gKOH	Polarization °V	
0.	- 4.95 (32°C)	
0.045	- 2.4	
0.09	- 2.4	
0.135	- 2.4	
0.18	- 2-1	- 1.75
0.22		
0.27	- 1.65	
0.36	- 1.5	
0.45	- 1.4	
0.54	- 1.0	
0.63	- 1.0	
0.67		- 1.00
0.9		- 0.6
1.12		- 0.6
1.35		- 0.7
1.8		- 0.7
2.25		- 0.6

The above results show that the reading of a 5 per cent invert sugar solution cannot be reduced to 0° ; the minimum levorotation is reached with about 2 per cent KOH (36° Be.) to 50 cc of sugar solution, stronger concentrations of alkali producing no apparent effect on the small residuary rotation.

With weaker invert sugar solutions the results were as follows:

TABLE VI

50 cc of invert sugar solution of various concentrations were heated as above with 1 cc of NaOH (40°Be) = 0.48g NaOH, decolorized, neutralized and made up to 55 cc.

% Invert Sugar	Polarization °V
5	- 1.6
4	- 1.2
3	- 0.8
2	- 0.6
1	- 0.3
0.5	- 0.3
0.25	- 0.1

The results in Table III obtained with raw sugars correspond to the weaker concentrations of invert sugar in Table VI; the error thus produced is not large enough to materially affect the results. The higher concentrations of invert sugar in Table VI correspond to the results obtained for molasses; the residuary rotation is large enough to introduce a serious error. A greater dilution of molasses to a reducing sugar content of about 1 per cent would involve a corresponding multiplication of a smaller error and lead ultimately to exactly the same result. To make effective use of the method it would be necessary to apply corrections to its results varying with the concentrations of reducing sugar. They would have to be fixed empirically for the concentrations of alkali employed at a certain temperature. Prolonged heating does not alter the results; the following table shows that the minimum of levorotation is reached after 15-20 minutes:

TABLE VII

50 cc of an invert sugar solution (about 5%) were heated with 0.45g KOH and on neutralization made up to 55 cc.

Time of Heating	Polarization °V
Not heated	- 4.9 (at 30°C)
15 min.	- 1.1
30 min.	- 1.0
45 min.	- 1.1
75 min.	- 1.0
105 min.	- 1.0
135 min.	- 1.0

The destruction of the rotary power of glucose also results in a slight levorotation; a minimum is apparently reached more easily than for fructose:

TABLE VIII

50 cc of 5% glucose, fructose and invert sugar solutions were heated with varying amount of alkali as follows:

gNaOH added	POLARIZATION °V		
	Glucose	Fructose	Invert Sugar
0.	+0.5	- 22.7	- 5.0
0.25	- 0.4	- 10.5	
0.12	- 0.4	- 3.1	
0.25	- 0.3	- 1.1	- 0.7
0.37	- 0.2	- 0.9	
0.50	- 0.2	- 0.7	- 0.7
0.75		- 0.6	- 0.5
1.00		- 0.6	- 0.4
1.25		- 0.6	- 0.4

An effect of the salts resulting from the neutralization of alkali on the saccharimeter reading of sucrose is hardly noticeable with quantities of alkali used in the method described above; but it becomes very apparent at higher concentrations:

TABLE IX

The following quantities of KOH were added to 50 cc of sucrose solution and the volumes completed to 55 cc.

gKOH	Polarization °V		
	Alkaline	Neutralized by SO_2	Neutralized by SO_2
0.		90.45	45.6
0.22			45.3
0.45			45.3
*0.67	87.30	90.05	
0.90			45.95
1.12	85.65	89.9	
1.35	84.35	89.3	
†1.8	82.75	89.5	

* Pellet's concentration.
† Cross' concentration.

Besides this effect due to the presence of neutral alkali salts a further decrease of the sucrose rotation is evidently due to the action of hot alkali (*). Thus heated (for 45 minutes) pure sucrose solutions acquire a yellow color, increasing with the amount of alkali. This coloration cannot result from the destruction of traces of invert sugar, as the polarization is noticably lower on neutralization, than that of a corresponding solution not heated.

TABLE X

The following amounts of alkali were added to 50 cc of sucrose solution and the volumes completed to 55 cc.

gNaOH added	Polarization °V			
	Not Heated		Heated	
	Alkaline	Neutralized (SO_2)	Alkaline	Neutralized (SO_2)
0.		45.41		
0.5	43.05	45.0	43.0	44.85
1.0	41.5	44 6		44.25
1.5	40.5	44.5	39.5	43.85
2.0	39.9	44 1	38.5	43.3

The explanation might be offered, that this difference is due to incomplete neutralization and the effect of alkali bicarbonate, which would result from a CO_2 content of the alkali with phenolphthalein as an indicator. The results were, however, not materially affected by a very distinct acidification. This acidity was not strong enough to cause inversion, the polarimeter reading remaining quite constant.

TABLE XI

The following quantitles of alkali were added to 50 cc of sucrose solution and on neutralization with acetic acid the volumes completed to 100 cc.

added gNaOH	Polarization °V				
	Not Heated Neutralized	Heated Neutralized	Not Heated Neutralized	Heated Neutralized	Acidified
0.	86.95	86.6			
0.33	86.85	86.0	61.2	61.0	61.1
0.67	86.6	85.3	61.1	60.85	60.8
1.0	85.85	84.55	60.9	60.6	60.5
1.33	85.75		60.75	60.25	60.05
1.67			60.8	60.15	60.0

Low results obtained by Cross' modification for pure sucrose could partially be explained by the influence of neutral alkali salts as the concentration of NaOH in his method is 2.5% NaOH; while Pellet's was only 1%.

A second correction would necessarily have to be applied in the application of these methods varying according to the concentration of alkali and sugar solution; this may be computed from the following tables:

TABLE XII

Varying amounts of alkali neutralized with SO_2 or CH_3COOH were added to 25 cc of sucrose solution and the volumes completed to 50 cc.

(*) Compare Herzfeld, Z. V. D. Zuckerindustrie, 43, 735.

added gNaOH	Neutral by SO Polarization °V	Decrease of rotation 100 °V=	Neutral by CH_3COOH Polarization °V	Decrease of rotation 100 °V=
0.	82.75			
0.3	82.25	99.43	82.60	99.82
0.6	81.90	98.97	82.35	99.52
0.9	81.65	98.67	82.10	99.23
1.2	81.20	98.13	81.95	99.03
1.5	80.75	97.58	81.90	98.97

TABLE XIII

25 cc of sucrose solutions of varying concentration are made up to 50 cc with and without 1.25g NaOH, neutralized by SO_2.

POLARIZATION °V		Decrease of rotation 100 °V =
Without Alkalisulfite	With Alkalisulfite	
101.65	99.8	98.2
61.8	60.75	98.3
40.8	40.1	98.3
19.6	19.2	98.0
10.2	10.0	98.0

Sodium acetate, resulting in Pellet's and Cross and Taggart's method from the neutralization of the alkali, has a weaker but still very noticeable effect on the sucrose rotation than sodium sulphite. Incomplete neutralization, leaving the solution alkaline would of course result in a very serious error, as shown in Table X. On the other hand, inversion on very strong acidification is not rapid enough to affect the results if the solution is not allowed to become warm and is polarized in a few minutes. On the contrary, a distinct increase in the saccharimeter reading is first noticed as Na_2SO_3 is being changed to $NaHSO_3$:

TABLE XIV

Equal volumes (25 cc) of a sucrose solution and an alkali sulfite solution of varying acidity (prepared by acidifying a solution of 12.5g NaOH in 250 cc with gaseous SO_2) were mixed and made up to the same volume.

Acidity of mixed sucrose solution 1 cc = cc $\frac{n}{100}$	Polarization °V
0.	80.6
0.8	80.6
2.8	80.6
15.9	80.8
19.7	80.9
31.0	81.0
36.0	81.0
39.5	80.95
0. (without alkali)	81.4
0.3	75.5
3.4	76.05
8.1	76.05
14.0	76.4
0. (without alkali)	76.8

The summary of the above experiments is that three sources of error are inherent in the direct methods of sucrose determination in the presence of reducing sugar:

(1) The residuary levorotation. This introduces a considerable error, especially with material high in reducing sugar and with methods using a weak concentration of alkali.

(2) Stronger concentration of alkali reduces this levorotation but introduces a second serious error, due to the decrease of sucrose rotation by the resulting alkali salts.

(3) Oxidation of sucrose by heating in alkaline solution, especially in the hydrogen peroxide methods.

Apparently correct results are occasionally obtainable due to a compensation of these errors.

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BATON ROUGE

THE COLORING MATTER OF CANE JUICES

BY

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The Coloring Matter of Cane Juices

A PRELIMINARY REPORT

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A process largely used in Louisiana and tropical sugar houses consists in adding small quantities (1 to 3 pounds per 1,000 gallons) of sodium phosphate to the clarified juice in the settling tanks. It is often claimed that this precipitates the soluble calcium salts. W. E. Cross conducted experiments showing that thus but a small fraction of these lime salts could be removed. (La. Bull. 144, pp. 16-18.) He used from 0.2 pound to 1.2 pounds of the salt per ton of cane, equivalent to .01g - .06g per 100 cc of juice for .132g CaO per 100 cc. Calculation alone shows that .565g of crystallized disodium-phosphate are therefore theoretically required. Cross' maximum amount is little more than 1/10 of this and removes in his experiments about 1/10 of the lime. The claimed precipitation of lime salts can, therefore, still less be expected by the amounts practically employed in the sugar houses, which are equal to the minimum used by Cross = .01g per 100 cc. Calcium phosphate is soluble in dilute acids, even acetic. Herewith does not agree the practice of using it at an acidity of about 1.0 cc n/100, while for complete precipitation alkalinity is required. That larger amounts under proper conditions will completely precipitate these lime salts also from cane juice needs no further proof; the following figures on the lime content of juices before and after treatment with sodium-phosphate obtained by titration with Clark's soap solution serve only as an illustration:

CLARIFIED JUICE.

Acidity 1 cc =	Alkalinity cc $n/_{100}$	mg Cao per 100 cc	Treatment, per 100 cc added:
...	0.1	117.5	Untreated
...	0.1	40.5	0.45 g Disodiumphosphate
...	1.0	10.8	0.45 g "
3.8	....	53.0	0.45 g " + 3.8 cc n H_3PO_4
3.6	....	32.5	large excess of " + 3.6 cc n H_3PO_4

.503g Disodium-phosphate are theoretically necessary for complete precipitation of 117.5 mg CaO at slight alkalinity. To effect the removal of lime in the clarified juices of the sugar house, about fifty times the quantity generally recommended would be needed, varying to some extent according to the lime content of the juices.

All that could be claimed for the above process is therefore the creation of a flocculent precipitate, which would tend to carry down fine turbidities not inclined to settle otherwise.

During these experiments it was noticed that the color of clarified juice treated with larger quantities of sodium phosphate was greatly improved. The change in color from the rather dark, blackish tint, which clarified juices acquire on cooling especially near neutrality, to a permanent bright yellow after calcium phosphate precipitation is very marked. The mere removal of soluble calcium salts could hardly produce this change. The addition of large amounts of calcium salts or magnesium salts, which latter are sometimes named as the cause of discoloration in juices (Prinsen Geerligs, Cane Sugar and Its Manufacture, p. 160), produced no further darkening of the liquors. We, therefore, sought the explanation in the removal of iron salt, which, during the heavy sulphitation, might pass into the juices from pipes and tanks. This suggestion occurred to us, especially when noticing the very striking improvement in color of a syrup clarified with sodium phosphate. This syrup, very dark, but quite clear when cold, turned very bright yellow and slightly turbid when brought to boiling. But every effort to separate this precipitate, suspected to be iron phosphate, by hot filtration resulted in a dark filtrate on cooling. Iron determination in the ash (after melting with potassium bisulfate) showed only a very insignificant amount of iron, as the juice had been concentrated in porcelain; and even in blackstrap, where the iron originally contained in the juice and on evaporation dissolved from pipes and effects would accumulate, it amounted to only .065% Fe. The addition of corresponding quantities of ferric salt to clarified juice did not materially increase its dark coloration. Ferric salt added to sucrose or glucose solution produces no similar darkening; therefore, amounts of iron glucosate corresponding to the above-mentioned traces of iron could not explain the dark color of juices. We therefore sought the explanation in an organic coloring matter, which might be carried down by the voluminous precipitate of calcium phosphate. In sugar refineries this clarification is generally practiced by addition of lime and phosphoric acid to the liquors in the "blowups." But much larger quantities would be necessary in sugar houses to take care of the larger amounts of coloring matter present in juices, and the cost becomes prohibitive. We consequently sought for a substitute method of creating a flocculent precipitant which would serve the purpose as well. After various experiments a precipitate of aluminum hydroxid produced by the addition of an alum (Newlands, Sugar, p. 585) salt and sodium hydroxid or milk of lime to very weak alkalinity of the juice (0.5 to 1.0 cc n/100) proved satisfactory. The precipitation of coloring matter seemed to be apparent from the yellowish-brown color of the precipitate. To avoid the introduction of SO_4-ions from the alum into the juice, which, together with the lime added, would cause very troublesome incrustations in the evaporators, we preferred the use of sodium aluminate, of which an addition less than 1/10 per cent of the weight of the juice is quite sufficient to produce a surprising change in color. The juice is heated to near boiling temperature, and the sodium aluminate, dissolved in little water, added. The precipitate, which, besides aluminum hydroxid, also contains lime, probably as calcium aluminate, settles well and is very easily and quickly filtered. The color of weakly alkaline filtrate is by far lighter than that of the original clarified juice. Reacidified to only 0.2 n/100 cc, the juice is of a light straw to lemon color, can be polarized directly without lead subacetate clarification, and remains light yellow indefinitely through concentration to syrup and massecuite, or when allowing the liquor to stand exposed to air for many days.

The process thus developed was to be tested on a larger scale in the Sugar Experiment Station sugar house, when the previously abandoned, but general recognized idea, that iron is in some unexplained manner responsible for the dark color of cane juices, cast doubt on the eventual success of the planned experiment, and caused us to test the influence of iron containers on syrup obtained by the above method. Nails, well cleaned with HCl and washed with water, were added to the syrup. It darkened within a few minutes. The amount of iron absorbed could only be exceedingly small, but by the Prussian blue reaction it could be easily shown that traces of iron had dissolved, while the light syrup gave an absolutely negative reaction. We determined the quantities sufficient to reproduce the dark color of ordinary sugar house liquors by adding to the yellow iron-free syrup varying amount of ferric salts; to each 33g of syrup 0.1 to 1.0 cc of a solution of 0.86g ferric ammonium sulfate in 100 cc. = 0.1 mg. to 1.0 mg. or .0003 to .003 per cent Fe. The lowest amount produced a noticeable darkening, the highest a dark brown coloration of the bright lemon-colored syrup. Sucrose, glucose and fructose solutions do not react in this manner.

Cane juice, therefore, contains another substance, which causes this reaction. The organic compounds characterized thereby belong to the polyphenols and phenol carbonic acids and usually on the basis of this very delicate iron reaction alone they have been identified in botanical researches as "tannins." But the chemical use of this term is more restricted. The occurrence of "tannin" or polyphenols in sugar cane is not at all surprising, as it is found in practiaclly all the higher plants, and, while its physiological role has as yet not been completely cleared, the researches of Moeller, Westermeyer, Kraus, etc., indicate that a certain relation exists between the formation of carbohydrates and tannin in plants. The hyaloplasma, according to Pfeffer and others, is impermeable for sugars in the free state and the migration of carbohydrates from cell to cell must therefore take place with the aid of other substances, the combinations with which are more easily translocated. As such compounds have been regarded the glucosides, especially of tannins. (v. Lippmann, Chemie der Zuckerarten, p. 1770.) Microscopic examination of the cane just before the harvest showed that the eyes, shoots and tops—*i. e.*, the assimilating parts—thus contain polyphenols distributed in the vascular bundles. In the leaves they are found in and near the bundles connected with the stomata; in the top joints of the stalk, where sugar is being accumulated, in the bundles as well as the parenchyma. In the lower joints, where the storage of sucrose has been practically completed, the bundles no longer give the iron reaction, while in the parenchyma the polyphenols occur sporadically in larger granules, adhering to the cell walls, or as contents of intercellular spaces. The latter fact would indicate a tendency to eliminate the polyphenols as a material which has now ceased to perform a necessary function in the process of sucrose accumulation.

This disappearance of the iron reaction could, however, be explained by a change of these polyphenols into a form which no longer gives this reaction. The so-called incrustating coloring matter of the fibrovascular bundles of cane and all other lignified plant tissues, especially of straw, wood, etc., is thus perhaps a physiological product of polyphenols. Tiemann and Haarman thought this to be coniferin, the glucoside of coniferylic alcohol, a polyphenol derivative which shows no iron reaction. But according to Czapek (Czapek, Biochemie der Pflanzen, Vol. I, pp. 567-9, and Vol. II, pp. 552 and 965), the characteristic reactions of wood with phenol, pyrogallol, orcin, thymol, phloroglucin, etc., and HCl do not exactly coincide with those of pure coniferin. He regards it as an aromatic aldehyde, related to coniferlylic alcohol, which he calls hadromal. It exists in the central lamellae of lignified cell walls in very firm combination, probably as an ester of lignin or cellulose. In agreement herewith is the experience of v. Lippmann, who isolated the coniferin by boiling 2½ tons of alcohol-extracted beet pulp with water for a very long time. The coniferin was so firmly retained that the phenol-HCl reaction did not disappear in the pulp after weeks of boiling. It is, therefore, not probable that the incrustating substance of cane fiber, although producing a very bright yellow color with lime or alkali, could pass into the cane juice to any extent, especially where alkalinity of the juice, containing fine bagasse, is avoided. But as it is colorless in neutral and acid solution and does not produce a dark color with iron, its presence would not be harmful.

Langguth-Steuerwald, however, mentions (International Sugar Journal, 1912, p. 53) this incrustating coloring matter of sugar cane as being possibly the substance causing in combination with iron the dark color of juices, molasses and sugars. He extracted it from bagasse with caustic soda and treated the extract with

Fehling solution (to eliminate the gums), but could not obtain it in crystallized form. He found it to be an aromatic compound, which yielded pyrogallol on dry distillation and catechol on fusion with potash. On heating with mineral acid it split off vanillin. It is evident that by alkaline extraction of bagasse, also, other polyphenols or ''tannins'' must be dissolved, especially from the eyes. The above reactions, particularly the one with iron, are, therefore, at least partially due to the latter, or to oxidation of the preparation by Fehling solution. The bundles of the cane stalks show the iron reaction after oxidation with alkaline copper solution or chromic acid mixture, which in the case of coniferin would produce vanillin; the latter gives an iron reaction. Langguth-Steuerwald thus sees a resemblance of his substance to phlobaphene, an oxidation product of tannins and polyphenols. He gave it the name ''saccharetin'' (also used in Prinsen-Geerlig's Practical White Sugar Manufacture, p. 7), which is apt to create the impression that this body is a particular constituent of sugar cane only, while, in fact, it is present in all lignified plant tissues. Czapek (Z. f. physiolog. Chemie, Vol. XXVII [1899], p. 154) obtained his above-mentioned hadromal in crystallized form from wood by treatment with stannous chlorid, extraction of the solution with benzol and purification by the way of its bisulfite compound; it gave all the lignin reactions in intensified form; only when heated it gave off an odor of vanillin. P. Klason (Schriften des Vereins der Zellstoff & Papierchemiker, Vol. II) regards it as a condensation product of coniferylic alcohol and oxyconiferylic alcohol. The color of clarified juice depends on its polyphenol content. In the cane as it goes to the mill the chief source of these are, as already mentioned, the eyes, especially if they have begun to bud. This fact can also be demonstrated microscopically by soaking bagasse in a solution of ferric salt, whereby preëminently the eyes will be stained black. A water extract of the eyes or young shoots gives a very strong greenish black iron reaction. Juice expressed from the (lower) internodes only is much lighter and juice from the nodes only much darker than the juice from the entire cane stalk.

Similar observations may be made also on other plants; thus the rudimentary leaves of the resting wheat germ also show the phenol iron reaction. The assumption of M. X. Sullivan (J. Ind. Eng. Chem. VI, p. 920) that vanillin is present in wheat germs does not, however, agree with their failure to give the red phloroglucin-HCl reaction characteristic of vanillin. The young tissues of the eyes and undeveloped leaves of sugar cane and the wheat seedling show, of the characteristic color reactions, of the older tissues, only the yellow color with alkali. The phloroglucin reaction does not appear until the leaves begin to assimilate and increases in intensity as the tissues grow older, just in inverse ratio to the iron reaction, the intensity of which gradually decreases and finally disappears with the age of the tissues. One might, therefore, rather assume that the same side-chain of the hadromal, which at first is responsible for the phenol-iron reaction, is gradually changed into a form which corresponds to the pholoroglucin-HCl, but no further to the former reaction.

The lower more mature joints of the cane, showing the phenol-iron reaction to a lesser degree than the immature top joints, produce therefore also a lighter colored juice than the latter. This is easily confirmed by an experiment, and is, therefore, another reason why the tops should be planted rather than ground for sugar production. The occurrence of polyphenols in sugar cane has been only casually mentioned in the literature, no importance being attached to the fact, in the problem of clarification.

W. Krueger, in Zuckerohr und seine Kultur in Java, mentions (page 152) the detection of tannin in sugar cane by Szymanski. C. A. Browne (La. Bull. 91, p. 9) notes the presence of tannic acid in the growing parts and in the peripheral region, especially near the buds and eyes, and (p. 27) he estimates .01 per cent "tannin, coloring matter, etc.," in the cane juice. On page 10 he explains that the well-known darkening of raw juices soon after expression is due to the action of an oxydase and quotes Bertrand, who has explained the darkening of vegetable tissues on exposure to the air by the action of an oxidizing enzyme upon various tannic bodies (Bull. 1896). But, after all, he does not draw the natural conclusion that the dark colored substance thus produced by oxidation is ferric tannate or another phenol-iron compound and that this fact has an important influence on the problem of clarification.

Prinsen-Geerligg asserts in Cane Sugar and Its Manufacture that: "A tannic acid of unknown composition in very minute quantities is present in young canes and in the green tops of ripe ones. The quantity is somewhat insignificant, and combines with albumen immediately after crushing, forming an insoluble precipitate; thus it does not interfere with the further stages of manufacture."

He explains, therefore, on pages 278-280, the dark coloration of some sugars, by an iron saccharate content, but is apparently at a loss to thus account for the color of blackstrap; he thinks here of caramel. In his latest book on Practical White Sugar Manufacture, he again emphasizes the darkening effect of ferric salt on juices due to formation of ferric saccharate (page 8).

But I have explained above that with the small quantities of iron found in juices, iron glucosate or saccharate could never account for such a deep black coloration. It is unmistakably the characteristic iron-phenol reaction, although the quantity of polyphenols present in clarified juice is naturally very small.

From the general occurrence of polyphenol-bodies in plants the presence of these substances should be expected also in the sugar beets, and indeed we find here a similar problem, which, like most facts concerning the beet sugar industry, has been more thoroughly investigated. (A summary on this subject is found in Wohryzek, Chemie der Zuckerindustrie, pp. 104-111.) The fast darkening of freshly expressed beet juice was noticed also here and the chromogenetic substance was shown to be catechol by Gonnermann and Grafe, who detected this polyphenol in beets. On expression of the juice catechol and ferrous salt react and rapidly form the black ferric compound by oxidation from the air or by an oxidase.

After the closing of the grinding season we have no material for the identification of the polyphenol present in sugar cane, but the color reaction of cane juice, greenish black with ferric chlorid and red on addition of sodium carbonate, speaks for catechol or one of its derivatives.

The estimation of these substances by the hide powder method, which, besides the tannins proper, absorb also the polyphenols and their derivatives, would hardly be feasible, but a colorimetric estimation would be applicable.

The means employed in the sugar industry to eliminate the dark color of juices, just explained by the presence of ferric polyphenol compounds, are characteristic reactions of these substances:

(1) Reduction to the colorless ferrous compounds.
(2) Decomposition by acidification.
(3) Decomposition by boiling temperatures.
(4) Decomposition of the ferric phenol compound by slight alkalinity precipitating iron.
(5) Elimination of polyphenols.

The methods 1 and 2 are extensively used in the cane sugar industry. By the action of sulphur dioxide the ferric-phenol compound is reduced as well as decomposed by the mere acidity of sulphurous acid, thus on sulphuring the raw juice. After the elimination of the most of this acid as calcium sulfite, oxidation by air, as well as mere neutralization, cause the return of the dark color. The similar decolorizing effect of other acids employed, as phosphoric, is due only to decomposition by the excess of acid. The acidity permissible for juices is too weak for complete decomposition at ordinary temperature, but its action may be intensified by heating. The liquors, light in color while running hot through the sugar house, darken on cooling and again become light on heating, due to alternate composition and regeneration of the ferric phenol compounds. This phenomenon is more apparent with acids other than SO_2, as then the darkening on cooling due to the restoration of ferric phenol is not retarded by incidental reduction. The above-mentioned (p. 3) very striking change of color of syrup containing phosphoric acid is thus explained. On cooling, the dark color reappears throughout the liquid while syrups acid by SO_2 darken only gradually from the surface on oxidation from the air. As a means of decolorization these methods are very inefficient and contact with air restores the ferric compound, resulting in a dark syrup, a black molasses and a sugar which gradually darkens on oxidation of adhering and occluded traces of phenol-iron. Such a sugar, apparently beautifully white when it leaves the centrifugal, can be easily tested for its keeping qualities if it is treated with NO_2 or Cl in a flask, when such traces will be restored to the ferric state immediately. The color of the oxidized and the fresh sample may then be compared by the Pekar method (Leach, Food Inspection and Analysis, page 317) as used in flour testing. In a very strange light appear, therefore, all those methods of clarification based on oxidation, employing Cl, H_2O_2 ozone, potassium permanganate, etc. (Prinsen-Geerligs, Cane Sugar and Its Manufacture, p. 184; Noel Deerr, Cane Sugar, p. 251.) Oxidation can only darken sugar house products containing ferrous phenol bodies. In fact, H_2O_2 is the proper reagent to detect their presence. The detection and approximate determination of traces of iron in juice and syrup can be effected by the aid of the Prussian blue reaction—that is, by acidifying and adding an excess of a mixture of ferro- and ferricyanide to react with either ferrous or ferric iron present, or by the sulphocyanate reaction after oxidizing with H_2O_2.

Owing to the sensitiveness of the phenol-iron reaction the dark color of cane juices reaches a maximum with extremely small amounts of iron present, while an increase above this maximum has no further effect on the color. The elimination of iron necessary to effect a permanent clarification must therefore not only be nearly, but absolutely, complete. The above tests reveal that such a complete precipitation of all traces of iron is effected by the described alumina clarification. As alkali salts of fixed organic acids and alkali saccharates prevent the precipitation of iron as hydroxides, phosphates, etc., by the formation of soluble complex iron salts, it is necessary to employ only the slightest excess of alkali. Aluminum hydroxide is much less soluble in sugar solutions than the iron hydroxides and carries down the traces of these which otherwise might remain in colloidal solution or might escape filtration. This reagent proved to be decidedly the most efficient of all precipitants used in these experiments. But the practical use of it in the sugar house is hardly possible in view of the recent strong advance in the price of aluminum salts, which has trebled during the last ten months. Sodium aluminate is obtained as an intermediate product in the manufacture of pure aluminum oxide from bauxite. The crude sodium aluminate liquor we used contained about 28% of solids, of which 6-7% were Al_2O_3, the rest NaOH and Na_2CO_3; it was of dark brown color, due to organic impurities, which did not, however, interfere with its use for our purpose. As it is not a commercial product, the price it would command lies entirely in the hands of the manufacturers and would likely be higher than that of other commercial aluminum salts. Other flocculent precipitates which, although less efficient, might be used for the same purpose are calcium silicate (which, however, would hardly be filtrable) or calcium carbonate. The warm (50°) clarified juice is limed to about 3-4 cc alkalinity, carbonated back to 0.5 cc and heated to boiling. Filtration is very quick (settling may be used instead); the filtrate is almost as free of iron as that from the alumina process. After resulphuring to acidity it will be necessary to filter off the deposited small quantities of calcium sulfite. This process is perhaps preferable to the alumina method, the drawback of which consists in the deposition on evaporation of small amounts of aluminum hydroxide remaining in the juice in the hydrosol form. The quantities of alkali introduced by the sodium aluminate are, however, no more harmful than the quantities of soluble lime salts it precipitates as calcium aluminate. On further thought, it is evident that the procedure just described corresponds to the double carbonatation process. By the large amounts of calcium saccharate the precipitation of iron is here prevented and takes places only when all the saccharate has been decomposed, just before reaching neutrality. If the carbonation is interrupted at this point (0.5 to 1.0 cc n/100 alkalinity), all iron passes into the precipitate. When carbonating in a single process directly to neutrality the slightest over-saturation would redissolve iron again. Herzfeld (who has investigated the behavior of iron salts in connection with the carbonation process) finds that ferrous salt is thus eliminated more easily than ferric salt, which fact he explains by the isomorphism of ferrous and calcium carbonates. (Z. V. Zuckerind [1895], p. 869; see also Rümpler, Nichtzuckerstoffe der Rüben, p. 463; Wohryzek, Chemie der Zuckerindustrie, p. 362.)

The elimination of iron undoubtedly accounts for the brighter juices usually obtained by the carbonatation process. This feature, as suggested above, could be just as well combined with the sulphitation process. But its advantage must, of course, be entirely lost again on evaporation in bodies of iron, which sucrose dissolves in neutral, in alkaline and especially in acid solutions. The process would lead to syrups of a permanent light yellow color if the liquors were not allowed to come into contact with iron again. White sugars equivalent to refined products and a very light molasses should thus be obtainable. Louisiana sugar houses operating less than two months could hardly be expected to go to the expense of a copper and brass installation. This may, however, be profitable where, as in the tropics, factories run for eight to ten months of the year, and where, as in Peru, copper installations are already in general use, such a process of iron elimination would be very inexpensive and advisable.

Heating cane juices even to boiling at .05 to .1 cc alkalinity produces no such dark color as usually is feared, provided the alkalinity is only temporary and concentration is effected after reacidification. A destruction of glucose is less noticeable by a loss of reducing power than by the fact that partial reduction of Fehling solution takes place even in the cold and by the appearance of a straw to lemon colored tinge of the juice. Among these decomposition products reducing Fehling solution are also phenols, such as pyrocatechin, protocatechuic acid (Hoppe-Seyler Z. phys. Chem., Vol. 13, 1899; see, also, Czapek, Biochemie der Pflanzen, Vol. I, p. 227; and glucinic acid, which also gives the black iron reaction of the phenols, although its constitution is not known. By heating at higher alkalinity invert sugar solutions are colored dark by glucinic acid, apoglucinic acid and other huminlike decomposition products. On further addition of an excess of milk of lime the color becomes lighter again, the akali salts of glucinic acid having a yellow color (v. Lippmann, Chemie der Zuckerarten, p. 330; also Jesser's researches, Wohryzek, p. 336). Ether extracts these phenolic decomposition products from the reacidified solution; they form a brown syrup from which needles crystallize. The aqueous solution of this substance gives a black reaction which turns to a much deeper brownish black on addition of sodium carbonate; it very strongly reduces Fehling solution. On prolonged heating the glucinic acid is largely destroyed, but the solution produces a dark iron reaction and a slight reduction of Fehling solution in the cold even then. We therefore have here a second source of polyphenol compounds in processes where the destruction of reducing sugar by heating with lime is practiced; that is, in the beet sugar carbonatation process and its copy in the cane sugar industry, the so-called Battelle process. Considerable amounts of glucinic acid may thus be formed, if the amount of lime added or the temperature of heating are not high enough; and as the lime salts of glucinic acid and its decomposition products are soluble and their reaction neutral, they remain in the juice, causing difficulties in evaporation, much more so, of course, in cane juices with the higher quantities of reducing sugar to be destroyed.

The danger to the color of juices arising from the formation of such polyphenol bodies is at first concealed by the precipitation of iron in the double carbonatation process. But the dark color must reappear gradually, as the metal is redissolved on evaporation in iron bodies. At the low temperatures of 50 degrees to 60 degrees C., usually observed in the cane juice carbonatation process, only small, but still noticeable, amounts of glucinic acid are formed in solutions of reducing sugars; to a minimum extent this is also the case in the above-suggested secondary carbonatation after a primary sulphitation. But if, as in some tropical countries (Cuba) cane juice is evaporated at distinct though slight alkalinity, considerable amounts of glucinic acid, up to over 7 per cent in cane molasses (v. Lippmann, Chemie der Zuckerarten, p. 330), and very dark products must result.

From these considerations it would thus appear best and advisable to avoid alkalinity of cane juices altogether, if iron evaporators, as usually the case, are to be used. The care employed in removing iron by double carbonatation or a similar process would not only be wasted, but more harm may be done by formation of glucinic acid, the amount of which is likely to be far in excess of the small quantities of phenol bodies naturally present in juices. The sulphitation in its present form hardly needs to be improved upon, unless one chooses to use a copper and brass installation. Lining of the iron bodies with rust-proof enamel might prove of some advantage, as it would keep down the iron content and might thus improve the color of the liquors. But the small quantities of iron naturally found in cane juice and the larger ones introduced with the lime are quite sufficient to produce fairly dark juices, and their elimination needs hardly to be attempted with an iron equipment, only partially protected by enamel.

An iron content of the liquors would, however, become comparatively harmless after elimination of the objectionable polyphenol bodies. Lead subacetate, the clarificant used in sugar analysis, is a precipitant of most polyphenols. Hide powder, specially prepared aluminum oxide and animal charcoal, used in the analysis of tanning materials, absorb, besides tannin, also all the other polyphenols. Only the charcoal process, some time ago discarded in the beet sugar industry, and now limited to refineries, is of practical value. Also vegetable charcoal processes (Norit, Eponit) have appeared; but whether they are as efficient or cheaper than the boneblack process is still an open question. The charcoal process might be of value in the syrup manufacture, where iron precipitation could not be attempted, as alkaline treatment destroys the flavor of the products. For readoption in white sugar houses the ordinary boneblack process is too expensive, but a simpler and more inexpensive modification would be the best and simplest solution of the problem. The destruction of the phenol bodies by oxidation, etc., is not feasible in the cane juice on account of the considerable quantities of reducing sugars present. The majority of such oxidation methods which were mentioned above, are devised rather for beet juices, which, after carbonatation, are quite free from reducing sugars, or for use in refineries after the glucose contained in the adhering molasses film has been removed by washing. Such processes consist in the use of hydrogen peroxide and barium peroxide (Stein and Crossfield), of ozonated air, which is blown through the limed juice (Steffen), of chlorine, the excess of which is removed by acetylene (Kittsee), of chloride of lime, etc. Wöstin (1868) applied lime treatment and carbonatation to the refining process; a similar process is patented in this country.

None of these chemical methods has, however, come into general use in refineries, although the quantities of polyphenols are here only a small fraction of those present in cane juices.

If a compromise between the direct production of the total output of a sugar house as a white product and refinery methods were to be made, the changes would likely be of a physical nature as careful affination of suitable, large-grained raw sugar, and reboiling of the mechanically filtered liquors; i. e., a method to restrict, but not to entirely eliminate, the use of boneblack.

CONCLUSIONS.

(1) The eyes and tops of sugar cane contain polyphenols which pass into the juice.

(2) Traces of iron are sufficient for the production of a very dark color in such juices.

(3) Reducing agents, such as SO_2 and hydrosulfites are only temporary remedies. The dark color returns on oxidation or cooling of juices and syrups.

(4) The dark shade of plantation white sugars, increasing in storage, is due to traces of phenol iron compound.

(5) A permanently light colored juice may be obtained by:

  (a) Complete elimination of all traces of iron and complete avoidance of contact of liquors with iron during further stages of manufacture.

  (b) Elimination of polyphenols by boneblack, vegetable charcoal, etc.

(6) The polyphenol content of juices could be reduced by topping cane low, using tops for planting. This would result in a distinct improvement of the color of juices.

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Louisiana State University and
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BATON ROUGE

TESTS OF THE

Power and Steam Consumption

OF

Sugar Factories

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TESTS OF THE POWER AND STEAM CONSUMPTION OF SUGAR FACTORIES.

INTRODUCTION.

In designing sugar factories that will give the maximum of fuel economy, it is important to have at hand data regarding the consumption of power and steam. An especially important item is the ratio of exhaust steam to total boiler steam. In fact, the question of the advisability of using pre-evaporators, vacuum juice heaters, and thermo-compressors depends upon this ratio. In other words, the size and arrangement of heating and evaporating apparatus—also the boiler plant—depends upon the amount of exhaust steam available. For example, it would not be advisable to use a quadruple effect where there is already an amount of exhaust steam in excess of that which would be required to operate a triple effect. There is a considerable difference of opinion as to the relative merits of different kinds of power distribution, such as direct steam drive, line shafting with centrifugal or power pumps driven by a central steam engine, direct electric motor drive, etc.

This bulletin contains data which has been secured during the last four years with the view of supplying information of the kind outlined above in so far as it has been possible. During the period of time mentioned this department has confined its experiments to two lines, namely, tests on heating and evaporating apparatus and tests on bagasse furnaces and boilers. Some of the results of the first line of work mentioned have been given in Louisiana Bulletins 138 and 149. In connection with these experiments, which were mostly of a research nature, it was comparatively easy to secure the data recorded in the present bulletin. The data in this bulletin were obtained at three typical, modern sugar factories, namely, the Adeline Sugar Factory Company, Adeline, Louisiana; the Guanica Central, Ensenada, Porto Rico; and Florida Central, Florida, Cuba.

The Adeline factory was designed to handle 1500 tons of cane per day to make white sugar and molasses of a grade suitable for table consumption. The sulphitation process of clarification is used. This system is practically the same as that commonly practiced in Louisiana, except that juices and syrup are filtered several times. In this respect the process is similar to that used in beet sugar manufacture. Most of the pumps in this factory are of the centrifugal type and operated through line shafting and belting from a Corliss engine, which also operated the centrifugals.

The capacity of Guanica Central is about 4500 tons per day, the product being raw sugar of 96 test. Most of the engines and pumps are direct steam driven, though a few of the pumps, the centrifugals, and the different conveyors are driven by electric motors.

Central Florida was designed to grind 1000 tons of cane per day, the product being 96 test raw sugar. All of the machinery, including the mills, in this house are driven by electric motors.

It may be well to state why these three particular factories were selected for these experiments. In the first place, such data is very difficult to obtain, and the expense is considerable. Few houses are arranged so that it is possible to secure such data under any circumstances. The three factories selected were all unusually well supplied with instruments and apparatus necessary for measuring the different quantities which it was necessary to measure. In addition to this, the management in each case was not only willing to furnish permission to carry on the experiments but assistance in preparing and making the tests as well.

The writer desires to acknowledge with thanks the coöperation of the managements of the three factories at which these data were secured. Among these may be mentioned in particular Mr. B. O. Sprague, superintendent of the Adeline Sugar Factory; Messrs. A. J. Grief and F. T. Maxwell, vice-president and general manager and superintendent of fabrication, respectively, at the Guanica Central; and Mr. William Bancroft, president of Dibert, Bancroft & Ross Company, which designed Florida Central and furnished most of its machinery.

EXPERIMENTS AT ADELINE.

OBJECT OF THE EXPERIMENTS.

The object of the tests at Adeline was to obtain data that would make it possible to construct a steam balance of the factory; that is, to determine the amount of steam generated in the boilers and the amounts of this steam used at the different stations throughout the factory.

FACTORY EQUIPMENT AND PROCESS.

As stated heretofore, the factory was designed to work 1500 tons of cane per 24 hours, though this capacity has seldom been attained in actual operation. The factory building has the shape of the letter "L," with the boiler room forming the L. The mill room is 68′ wide x 112′ long. The boiler room is 68′ by 154′. The clarification house is 80′ wide x 120′ long and contains two galleries along one side of the building and across the end, one above the other, and a small stage at one end above the top gallery. The boiling house is 80′ wide x 56′ long x 48′ high to the eaves. The boiling house has two full floors above the ground floor and a gallery for tanks and for supporting a sugar storage hopper. The distance from the boilers to the vacuum pans is approximately 260′, to the evaporators 220′, and to the heaters 120′. The cane is supplied to the mill carrier by means of a Walsh rake and three cane derricks of the grab type. These hoists are approximately 350′ from the boilers. The distance is so great that the steam from the hoisting engines is exhausted to the atmosphere, no attempt being made to conduct it back to the factory and use it.

As stated, the sulphitation process is used with several filtrations of juice and syrup. Figure 1 shows graphically the principal steps of the process. Figure 2 shows the method of handling condensation and returning it to the boilers. There are four boiler feed pumps, three of which are of the automatic receiver type, the other without a receiver. There are two vacuum pans, the condensation from which is removed by two of these automatic receiver pumps. One of the raw juice heaters operates with live steam and the other with exhaust steam. A large por-

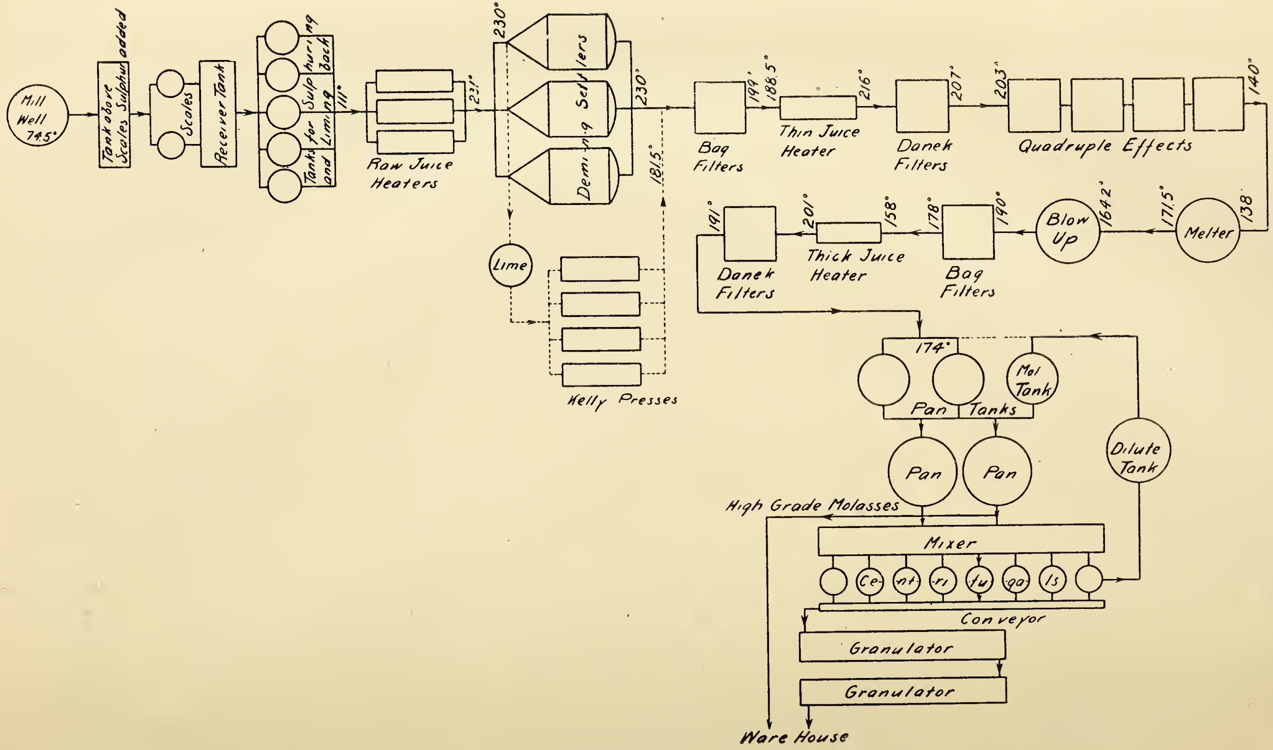

Mill Well 74.5°
Tank above Scales Sulphur added
Scales
Receiver Tank
Tanks for Sulphuring and Liming back
111°
Raw Juice Heaters
231°
230°
Demming Settlers
230°
181.5°
Lime
Kelly Presses
Bag Filters
199°
188.5°
Thin Juice Heater
216°
Danek Filters
207°
203°
Quadruple Effects
140°
138
Melter
171.5°
164.2°
Blow Up
190°
Bag Filters
178°
158°
Thick Juice Heater
201°
Danek Filters
191°
174°
Pan Tanks
Mol Tank
Dilute Tank
Pan
Pan
High Grade Molasses
Mixer
Ce nt ri fu ga ls
Conveyor
Granulator
Granulator
Ware House

Fig. 1

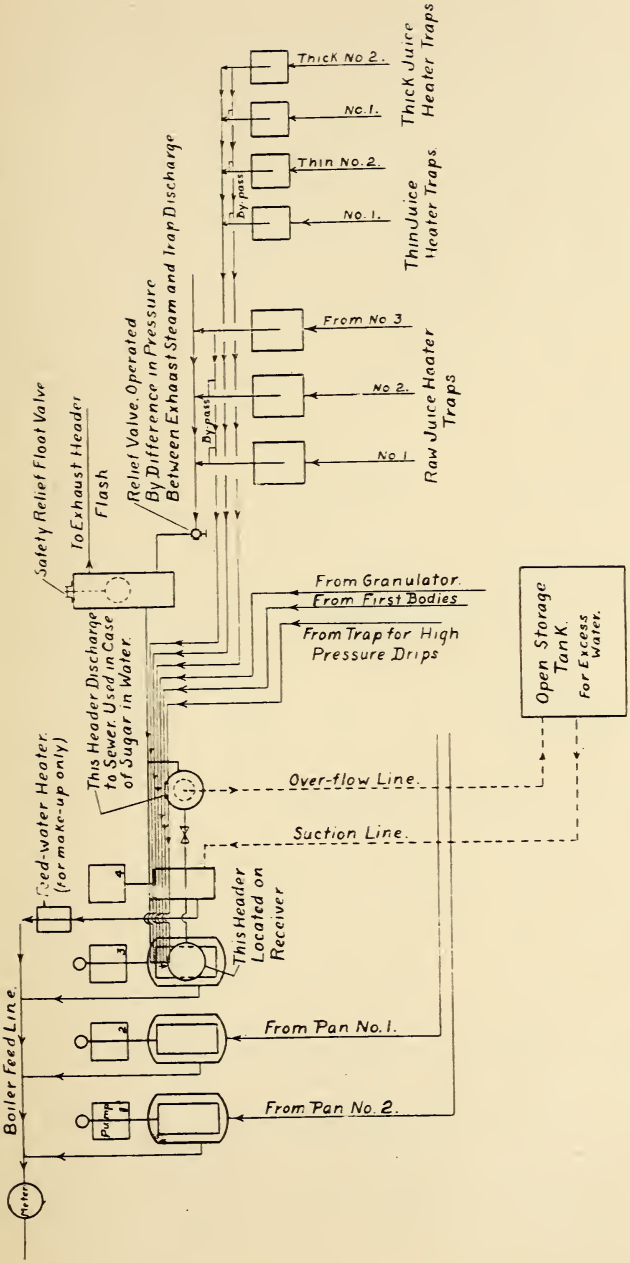

Thick No 2.
No. 1.
Thin No. 2.
No. 1.
Thick Juice Heater Traps
Thin Juice Heater Traps
By-pass
From No 3
No 2.
No 1
Raw Juice Heater Traps
By-pass
Relief Valve, Operated By Difference in Pressure Between Exhaust Steam and Trap Discharge
Safety Relief Float Valve
To Exhaust Header
Flash
From Granulator.
From First Bodies
From Trap for High Pressure Drips
Open Storage Tank. For Excess Water.
This Header Discharge to Sewer. Used in Case of Sugar in Water.
Over-flow Line.
Suction Line.
Feed-water Heater. (for make-up only)
4
3
2
Pump 1
This Header Located on Receiver
From Pan No. 1.
From Pan No. 2.
Boiler Feed Line.
Meter

Fig. 2

tion of the feed water naturally comes from these raw juice heaters. There are also two thin juice and two thick juice heaters, though only one of each is operated at one time under normal running conditions. Automatic receiver pump, No. 3, handles all of the condensation except that from the two vacuum pans. This includes condensation from the raw juice heaters, the thin juice heaters, the thick juice heaters, the steam engine separators, and the first bodies of the evaporators; also the drips from the high pressure steam lines. Each of these different lines drains into a trap and the traps discharge through separate lines into the header above the receiver of pump No. 3. The traps for the raw juice heaters are located on the ground floor below the level of the header, each trap having a live steam connection for discharging.

In order to provide for flash from the vapors coming from the condensation leaving the live steam raw juice heaters, a flash drum is provided as shown. The raw juice heaters are so connected that condensation from the exhaust heater can always be by-passed around the trap directly to the header. The condensation from the live steam heater passes through the relief valve into the flash drum and from there to the header, where it mixes with the condensation from other sources. From the top of this flash drum a flash connection is made to the exhaust steam mains. At one end of this drum there is an outlet with float-operated valve so that water can not rise above a certain height in the drum. This prevents water from getting into the exhaust main. By means of this drum all disturbances of the kind mentioned above are prevented, the liberated flash being utilized along with the exhaust steam in the evaporators and other apparatus using exhaust steam. The make-up feed water is handled by pump No. 4, which draws from an open tank outside of the boiler room. The water in this tank is condensation coming from the second and third bodies of the evaporators. There is also an overflow into this tank from the header described above, which operates occasionally when the supply of condensation is greater than the boiler feed requirements at the time. This make-up feed water, coming from the tank, passes through a closed feed water heater heated by live steam.

For a list of the principal apparatus contained in the factory, see Appendix A. The two 13′ vacuum pans of the express type are operated with an average steam pressure of 30 pounds gauge, requiring live steam most of the time. However, at the beginning of a strike, exhaust steam is occasionally used. There are no direct acting steam pumps in the factory with the exception of boiler feed pumps and a few others, the majority of the pumps being of the centrifugal type operated through belting and line shafting from a single Corliss engine. The water supply for the factory is taken from Bayou Teche, which is approximately 150′ from the boiling house where the condensers are located. The lift from the surface of the water in the bayou to the ground floor of the factory is approximately 10 feet. The water is pumped into a standpipe 5′ in diameter by means o fa 12″ centrifugal pump, driven from the line shaft heretofore mentioned and lifted a height of approximately 60′ above the ground floor. This standpipe supplies injection water to the condensing system, this condensing system being of a special central type. Each of the quadruple effects and vacuum pans is served by an individual condenser, but the air pipes from each condenser are connected to a central condenser, from which a single vacuum pump of the rotative dry vacuum type pumps the air and other incondensable gases. Each individual condenser has its own injection water supply under control.

All steam pipes and containers in the factory are well covered with insulating material. A large portion of the hot liquid pipes and containers, however, are uncovered. On account of the large number of filtrations with intermediate reheatings, also on account of the large amount of remelting, the steam consumption with this process is considerably greater than with the process commonly practiced in Louisiana.

PROCEDURE IN MAKING THE EXPERIMENTS.

The principal steam consuming stations in the house are as follows: (1) raw juice heaters; (2) thin juice heaters; (3) thick juice heaters; (4) feed water heater; (5) quadruple effects; (6) vacuum pans; (7) granulators; (8) cane hoisting engines; (9)

syrup blow-ups; (10) syrup melters; (11) molasses melters; (12) mud defecators; (13) steam power; (14) radiation and unaccounted.

The general procedure in making the steam balance was to determine (a) the total steam generated by means of boiler tests; (b) the steam consumed at stations 1 to 14, inclusive; (c) subtracting the steam consumed (b) from the steam generated (a) to get radiation and unaccounted for (c). In determining (a) the feed water was measured by means of a recording Venturimeter which was a permanent part of the factory equipment. The steam pressures and feed water temperatures were found by means of recording apparatus, also part of the factory equipment. The steam quality was determined by means of a special throttling calorimeter. In determining items 1 to 7, inclusive, of (b) the thermal efficiency in percent was first found in each case by one or more individual tests, in which the condensation was weighed and pressures, temperatures and other necessary observations made. The results of many of these individual tests are given in Louisiana Bulletin 149. Next, the weight of the product handled by each apparatus per ton of cane was found by weighing or by calculation. With these data known, the heat required and the corresponding weight of steam was calculated. The steam consumption for items 8 to 12, inclusive, was determined directly by means of steam orifices. Item 13 (steam for power purposes) was found through I. H. P. tests of the steam engines and pumps in the house. With the total I. H. P. known, the equivalent heat (B. T. U.) and weight of the boiler steam were calculated. The tests upon which the steam balance was based covered the period from 7 a. m., December 2, 1915, to 11 a. m., December 11, 1915. However, some of the individual tests for thermal efficiency and steam consumption per unit of work done were made during the 1911 and 1913 seasons. During the period of the tests a record was also made of the oil fuel burned, which gave the data necessary for calculating the fuel cost at each station.

All instruments, such as thermometers, pressure gauges, vacuum gauges, etc., used in the experiments were carefully calibrated. The temperatures referred to in this bulletin are in all cases according to the Fahrenheit scale.

Steam Balance.

Period covered, from 7 a. m., December 2, 1915, to 11 a. m., December 11, 1915.

Total time, hours	210.75
Total cane ground, tons	12,144.58
Cane ground per hour, tons	57.62
Cane ground per 24 hours, tons	1,383

RAW JUICE HEATERS.

Total mixed juice, tons (juice scales)	11,188.23
Mixed juice per ton of cane, tons	.9213
Mixed juice per ton of cane, lbs.	1,842.6
Mixed juice per 24 hours, tons	1,274.17
Temperature juice entering raw juice heaters	82
Temperature juice leaving raw juice heaters	201
Heat added = (201 — 82) × .914 × 2,548,340 = (B. T. Us. per 24 hrs.)	277,182,941.8
(B. T. Us. per hr.) 277,182,941.8 ÷ 24	11,549,289
Equivalent lbs. water F. & A. 212°, 11,549,289 ÷ 970.4	11,901
Efficiency (Thermal) of raw juice heaters, % (from individual tests)	95
Steam F. & A. 212° (actual), lbs. $\frac{11901}{.95} =$	12,527.9
Steam F. & A. 212° (actual), % on cane	10.87
Equivalent steam at boiler pressure of 100 lbs. gage, feed water temperature 231° and steam quality 96% (boiler conditions) $\frac{10.87}{1.02} =$	10.66%

Factor of evaporation, $\frac{1188.8 - 199.2}{970.4} = \frac{989.6}{970.4} = 1.02$

THIN JUICE HEATERS.

Bx. of mixed juice	13.19
Bx. of thin juice	13.42
Mud in mixed juice, lbs. per ton of cane (by test)	22.23
Total mud per day, lbs., 1383 × 22.23	30,744
Total mud per day, tons	15.372
Mixed juice, less mud tons, 1272 — 15.372	1,258.8
Water lost, % on mixed juice (without mud) $\frac{13.42 - 13.19}{13.42} =$	1.714
Water lost tons, 1258.8 × .01714 =	21.58
Thin juice per day, tons = 1258.8 — 21.58 =	1.237.22
Thin juice % on cane, $\frac{1237.22 \times 100}{1383.01} =$	89.46
Specific heat corresponding to 13.42 Bx	.912

Temperature juice entering thin juice heater	180
Temperature juice leaving thin juice heater	203
Heat added B. T. U. per 24 hrs. $(203 - 180) \times .912 \times 1237.22 \times 2000 =$	51,924,829
Heat added per hr. B. T. U	2,163,534
Equivalent steam F. & A. 212°, lbs. $\frac{2,163,534.5}{970.4} = 2229.5$	
Thermal efficiency, % (from individual tests)	92
Steam F. & A. actually required, lbs. $\frac{2229.5}{.92} =$	2,423.4
Equivalent steam F. & A., actually required, % on cane	2.103
Equivalent steam, boiler conditions, % on cane, $\frac{2.103}{1.02} =$	2.06

THICK JUICE HEATERS.

Brix of evaporator thick juice	56.21
Brix thin juice	13.42
% Evaporation $= \frac{56.21 - 13.42}{56.21} =$	76.13
Water evaporated in quads, tons per day, $1237.2 \times .7613 =$	941.8
Thick juice, tons per days, $1237.2 - 941.8 =$	295.4
Thick juice, % on cane $= \frac{295.4}{1383}$	21.36
Specific heat, thick juice	.633
Temperature juice entering	132.1
Temperature juice leaving	207
Heat added, B. T. U. per 24 hrs., $(207 - 132.1) \times .633 \times 295.4 \times 2000 =$	28,048,230
Heat added, per hr., B. T. U	1,168,676
Equivalent of heat added, lbs. steam F. & A. 212° per hr., $\frac{1,168,676}{970.4} =$	1,204
Efficiency % (by individual test)	82
Steam F. & A. 212° actually required, lbs. per hr., $\frac{1204}{82} =$	1,468
Equivalent steam F. & A. 212° actually required, % on cane.	1.274
Equivalent, steam, boiler conditions, % on cane	1.249

QUADRUPLE EFFECT.

Thin juice, tons per day	1,237.22
Thick juice, tons per day	295.40
Water evaporated, tons per day	941.82
Water evaporated per lb. of steam, lbs. (by test)	3.637
Steam pressure, 1st Calandria, lbs., gage	3.60
Steam per day, lbs. $= \frac{941.82 \times 2000}{3.637} =$	517,824

Steam per day, tons, $= \frac{517,824}{2000} =$	258.912
Steam, % on cane $= \frac{258.912}{1383} =$	18.72
Steam, F. & A. 212°, % on cane, $18.72 \times \frac{963}{970.4} =$	18.57
Equivalent steam, boiler conditions, $\frac{18.57}{1.02} =$	18.21

Vacuum Pans.

Brix of standard syrup (calculation)	52.2
% decrease in water content $\frac{52.2 - 13.19}{52.2} =$	74.7
Decrease in water content, tons per day, 1258.8 × .7473	940.71
Standard syrup, tons per day, 1258.8 — 940.7	318.1
Standard syrup, % on cane, $\frac{318.1}{1383} =$	23.00

Molasses (actual measurement).

Molasses, tons during period	157.35
Molasses, per ton of cane, tons, $\frac{157.35}{12,144.58} =$	.0129
Molasses, per day, tons, 1383 × .0129 =	17.92
Wash, tons during period (actual measurement)	1,230.1
Wash per ton of cane, tons, $\frac{1,230.1}{12,144.58} =$	.10128
Wash per day, tons, 1383.01 × .10128 =	140.07
Brix of massecuite	95.5
Brix of standard syrup	52.2
% Evaporation $= \frac{95.5 - 52.2}{95.5} =$	45.34
Water evaporated, tons per day, 318.1 × 4534	144.3
Massecuite per day from syrup, tons, 318.1 — 144.3 =	173.8
Brix of molasses	76.5
% Evaporation $= \frac{95.5 - 76.5}{95.5} =$	19.90
Water evaporated, tons per day = 17.92 × .99	3.566
Massecuite per day from molasses, tons, 17.92 — 3.57 =	14.35
Brix of wash	75.0
% Evaporation $\frac{95.5 - 75.0}{95.5} =$	21.47

Water evaporated, tons per day, 140.07 × .2147	30.07
Massecuite per day from wash, tons, 140.07 — 30.07 =	110.00
Total massecuite per day, tons, 173.8 + 14.35 + 110.0 =	298.15
Massecuite per ton of cane, tons, $\frac{298.15}{1383} =$	.2156
Massecuite, % on cane	21.56
Total syrup, molasses and wash per day, tons, 318.1 + 17.92 + 140.07 =	476.09
Total massecuite per day, tons	298.15
Total water evaporated in pans per day, tons	177.94
Water evaporated per lb. of steam, lbs. (by individual tests)	0.85
Steam per day, tons, $\frac{177.94}{0.85} =$	209.34
Steam, % weight on cane, $\frac{209.34}{1383} =$	15.14
Equivalent steam F. & A. 212° % on cane, $\frac{15.14 \times 928.15}{970.4} =$	14.48
Equivalent steam, boiler conditions, $\frac{14.48}{1.02} =$	14.20

GRANULATOR.

Condensed steam on raw juice, %	.572
Condensed steam on cane, % .572 × .9213 =	.527
Steam pressure, lbs., gage	71.0
Steam F. & A. 212°, % cane, $\frac{.527 \times 896.6}{970.4} =$	.49
Equivalent steam, boiler conditions, %, $\frac{.49}{1.02} =$	.48

FEED WATER HEATER.

Steam condensed per 24 hours (by individual test), lbs	74,385.0
Steam condensed per 24 hours (by individual test), tons	37.19
Pressure of steam, lbs., gage	11.7
Steam F. & A. 212°, tons, $\frac{37.19 \times 950}{970.4} =$	36.41
Steam F. & A. 212°, % on cane, $\frac{36.41}{1383} =$	2.63
Equivalent, steam, boiler conditions, % on cane, $\frac{2.63}{1.02} =$	2.58

STEAM ORIFICES.

Table 1 gives the steam consumption at a number of stations as determined by means of steam orifices. The orifices were in nearly all cases about ¼″ long with sharp edges. Observations of the steam pressure entering and leaving were made and the steam flow per hour determined by means of the formula:

$$F = \frac{C\ A\ P^{.97}}{60\sqrt{x}} \quad \text{where}$$

C = A constant, the value of which depends upon the ratio of initial to final pressure.
A = Area of orifice, sq. in.
P = Initial absolute pressure of steam, lbs. per sq. in.
x = Quality of steam = .96.

The values of C were determined by methods and data given in Moyer's Steam Turbines and Thomas' Steam Turbines.

TABLE 1.

STEAM MEASURED BY MEANS OF ORIFICES.

STATION	Initial Pressure, lbs. Gage	Exit Pressure, lbs. Gage	Diameter of Orifice, Inches	Value of C	Value of A	Value of F	Weight of Boiler Steam, % on Cane
Mud Defecators....	92.1	82.0	9/16	.539	.248	773	0.67
Syrup Blow-ups....	89.1	74.1	9/16	.644	.248	925	0.80
Thick Juice Melters	95.2	90.0	1/2	.382	.196	318	0.34
Molasses Melters...	96.5	94.8	1/2	.28	.196	318	0.28
Oil Burners........	....	....	....	....	....	1347	1.16
Cane Handling Machinery........	95.4	84.8	1.0	.539	.785	2448	2.16

TABLE 2.

MECHANICAL ENERGY.

I. H. P., Steam Engines and Pumps.

Crusher..	48.55
First, second and third mills..............................	377.64
Fourth mill	135.72
Vacuum pump..	55.10
Centrifugals, centrifugal pumps, etc......................	255.04
Bagasse drag..	5.21
Raw juice pump..	16.00
Mill well pump..	6.18
Boiler feed pumps.......................................	7.82
Total I. H. P..................................	907.26

Total work, ft. lbs. per minute, 907.26 × 33,000 = ...	29,939,580.0
Total work, ft. lbs. per hour, 29,939,580 × 60 = ...	1,796,374,800
Equivalent heat B. T. U. per hour, 1,796,374,800 ÷ 778.0 =	2,308,965
Equivalent steam F. & A. per hour, lbs., 2,308,965 ÷ 970.4 =	2,379.4
Equivalent steam F. & A., % on cane..........................	2.06
Equivalent steam, boiler conditions, % on cane, $\frac{2.06}{1.02}$ = ...	2.02

BOILER FEED WATER.

Feed water per hour lbs. (Venturimeter)..................	80,295.6
Feed water, per 24 hours, lbs., 81,295.6 × 24 = ...	1,951,094.4
Feed water per 24 hours, tons, $\frac{1,951,094.4}{2000}$ = ...	975.5
Feed water, % on cane, $\frac{975.5}{1383}$ = ...	70.538
Average boiler pressure, lbs. gage..........................	100.00
Average feed water temperature..........................	231.00
Boiler H. P. = $\frac{.7053 \times 1.02 \times 1383 \times 2000}{24 \times 34.5}$ = ...	2,420
Boiler H. P. per ton of cane per 24 hours, $\frac{2420}{1383}$ = ...	1.74

Summary.

TABLE 3.

ADELINE STEAM BALANCE.

	Steam, % on Cane	Steam, % on Total	Boiler H. P.
Boilers..................................	70.54	100	2420.0
Raw juice heaters........................	10.66	15.10	365.0
Thin juice heaters........................	2.06	2.92	71.0
Thick juice heaters........................	1.25	1.77	43.0
Quads......................................	18.21	25.82	625.0
Vacuum pans................................	14.20	20.13	486.0
Syrup blow-ups.............................	0.80	1.13	27.0
Granulators	0.48	.68	17.0
Oil burners................................	1.16	1.65	40.0
Cane handling machinery....................	2.16	3.06	74.0
Syrup melters..............................	0.34	.48	12.0
Molasses melters...........................	0.28	.40	10.0
Mud defecators.............................	0.67	.95	23.0
Feed water heaters.........................	2.58	3.66	89.0
Mechanical work............................	2.02	2.86	69.0
Radiation and unaccounted..................	13.67	19.39	469.0

FUEL COSTS.

Table 4 gives the estimated fuel cost to be charged to each of the stations, including radiation. The following shows the method used for calculating the fuel cost charged to the raw juice heaters. The same method was used for the other stations:

Oil used, bbls	1230.87
Oil used, gals. per ton cane, $\frac{1230.87 \times 42}{12{,}144.6} =$	4.26
Price of oil per bbl. (assumed), dollars	1.00
Steam consumed by raw juice heaters, % on boiler steam (Table 3)	15.10
Equivalent oil, raw juice heaters, bbls., .151 × 1230.87 =	186.00
Equivalent oil, raw juice heaters, in dollars, 186.00 × 1.00 =	186.00
Total radiation loss in factory, % on boiler steam	19.39
Equivalent oil, bbls., 1230.87 × .1939 =	238.54
Radiation loss charged to raw juice heaters, bbls. of oil, $\frac{15.1}{(100 - 19.38)} \times 238.54$	44.70
Value of oil charged to heaters, dollars, = 186.00 + 44.70 =	230.70
Value of oil consumed by heaters per 1000 tons cane, $\frac{230.7}{12.144} =$	$18.99

TABLE 4.

FUEL COSTS.

	Fuel Cost per 1000 Tons of Cane
Raw juice heaters	$ 18.99
Thin juice heaters	3.68
Thick juice heaters	2.23
Quadruple effects	32.47
Vacuum pans	25.32
Syrup blow-ups	1.43
Granulator	0.86
Oil burners	2.07
Cane handling machinery	3.85
Syrup melters	0.61
Molasses melters	0.50
Mud defecators	1.20
Feed water heaters	4.56
Mechanical energy	3.69
Total	$101.46

HEAT LOSSES FROM HOT LIQUID LINES AND CONTAINERS.

During the 1911 season, observations were made at Adeline covering a period of 24 hours, the object of which was to secure data from which the heat losses, including radiation from liquid lines and containers, could be calculated. The calculation of these heat losses was based on the weight of the product handled at each station and the loss in temperature between any two points. The temperature observations made during the 24 hour period are shown on the process diagram, Figure 1. The weights of products involved were for the operating conditions of the 1911 season and are therefore different from those of 1915 used in making out the heat balance given above.

To show the method of calculating the heat losses, the following calculation for the loss of heat between the thin juice heaters and leaving the Danek filters, is given. Table 5 gives the results of the observations therefrom.

TABLE 5.

HEAT LOSSES FROM LIQUID LINES AND CONTAINERS.
(Adeline.)

	Loss in Temperature	Equivalent Steam at Boiler, % Wt. of Cane	B. H. P.
Loss of heat from thin juice between Deming separators and leaving bag filters	31.0	2.59	82.6
Loss of heat between thin juice heaters and leaving Danek filters	9.0	.675	21.5
Radiation loss in pipe line between Danek filters and quads	4.0	.300	9.6
Radiation loss in thick juice bag filters	12.0	.209	6.7
Loss between bag filters and syrup heater	20.0	.352	11.2
Loss between thick juice heater and leaving thick juice Danek filters	10.0	.176	5.6
Loss between quads and melters	2.0	.036	1.2
Loss between melters and blow-ups	7.3	.128	4.1
Radiation loss between thick juice Daneks and vacuum pans	17.0	.300	9.6
Totals	......	4.76	151.9

It may be well to call special attention to the first item in the above table, namely, the loss of heat from the thin juice between the Deming separators and leaving the bag filters. It will be noted that the temperature drop was 31°. This drop was not due to radiation but to the reduced pressure of the hot juice on leaving the closed Deming separators. In other words, when the juice under pressure was released into the bag filters, the temperature dropped to 212°, and a loss of heat through vaporization occurred. By referring to the heat balance heretofore given it will be noted that this loss was avoided by heating the juice in the raw juice heaters to 212° or less.

EXPERIMENTS AT GUANICA CENTRAL.

OBJECT OF THE EXPERIMENTS.

The object of the experiments at Guanica was similar to that at Adeline, except that the steam consumed only could be determined. On account of the particular conditions it was not possible to measure the steam generated by the boilers.

FACTORY EQUIPMENT AND PROCESS.

As stated heretofore, the factory is supposed to handle about 4500 tons of cane per day when operating normally. There are two main departments in the factory organization, namely, the engineering department and the fabrication department, the milling plant being under the control of the former. The products of the factory are 96 test raw sugar and low molasses. The clarification system is of the closed Deming type. Figure 3 is a flow diagram which illustrates the process. It will be noted that three grades of massecuite are boiled, which are termed straight strikes, mixed strikes, and crystallizer strikes. Figure 4 is a diagram of the method of handling hot condensation and returning same to the boilers. The vacuum pans operate under pressures of 80 to 100 pounds gauge, the evaporators under pressures of approximately 3 to 5 pounds gauge, the exhaust heaters under a pressure of about one pound gauge, and the live steam heaters under a pressure of about 12 pounds gauge. All of the condensation from these different sources used for feed water is delivered to a boiler feed receiver, from which hand-controlled direct-acting feed pumps deliver it to the boilers. The quadruple effects and the vacuum pans are situated some 40 feet above the boiler feed receiver. From the top of the boiler feed receiver there is a vapor line connecting to the exhaust main. In this line there is a relief valve set at 15 pounds gauge, so that there will be a pressure of 15 pounds gauge in the receiver. The condensation from the vacuum pans is discharged by traps into an auxiliary closed receiver with a vapor connection to the feed water heater used for heating make-up feed water. The condensation is discharged by gravity into the boiler feed receiver. The condensation from live steam heaters is discharged, by traps, directly to

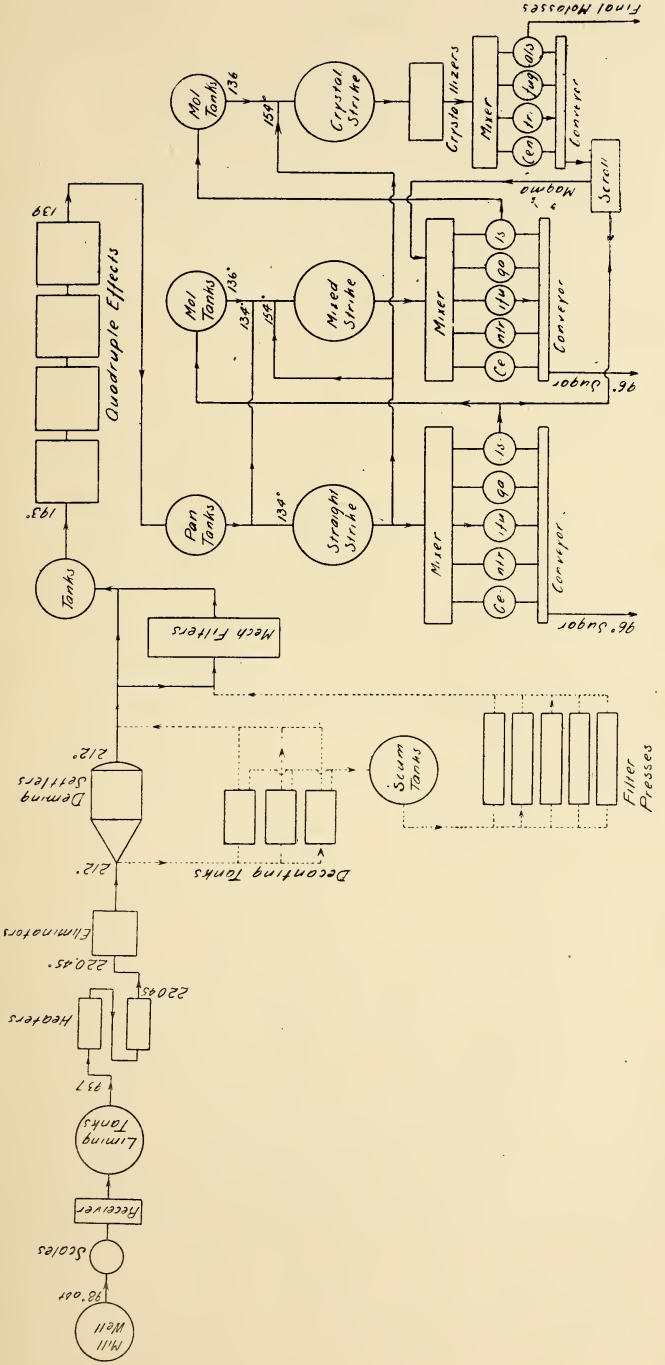

Mill Well
98.08t
Scales
Receiver
Liming Tanks
937
Heaters
220.45
220.45°
Eliminators
212°
Deming Settlers
212°
Decanting Tanks
Scum Tanks
Filter Presses
Mech Filters
Tanks
193°
Quadruple Effects
139
Pan Tanks
134°
Straight Strike
Mixer
Ce ntr ifu ga ls
Conveyor
96° Sugar
Mol Tanks
136°
134°
154°
Mixed Strike
Mixer
Ce ntr ifu ga ls
Conveyor
96° Sugar
Magma
Scroll
Mol Tanks
136
154°
Crystal Strike
Crystallizers
Mixer
Cen tri fug als
Conveyor
Final Molasses

Fig. 3

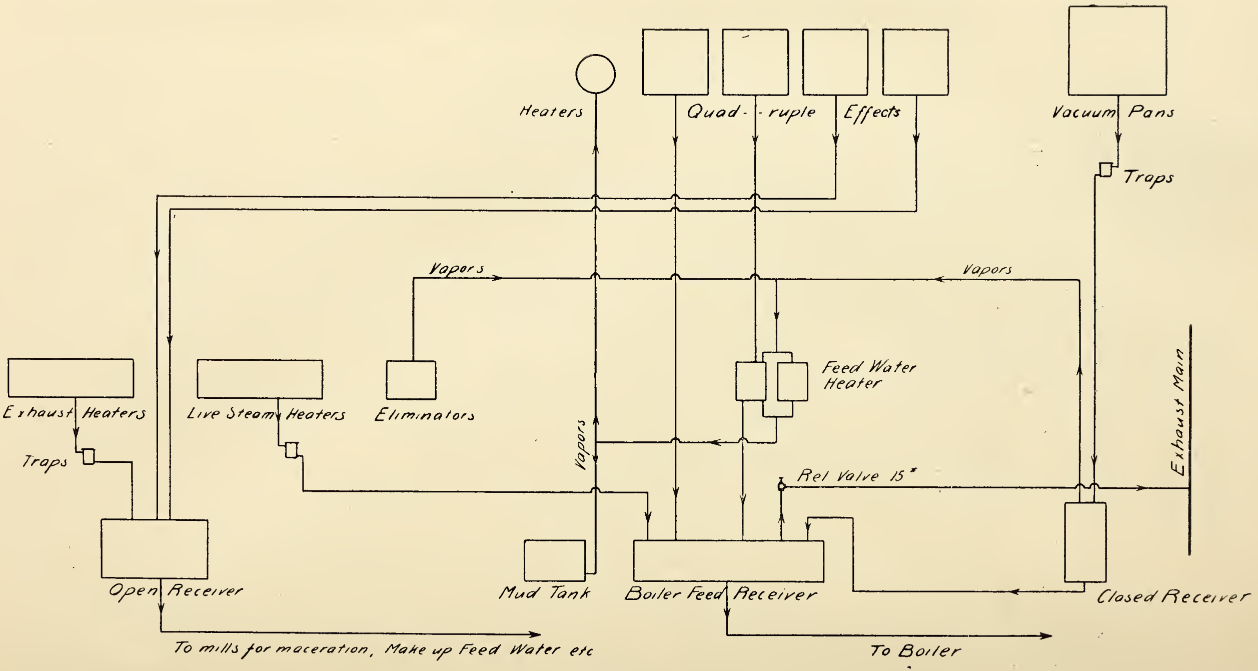

Heaters
Quad- -ruple Effects
Vacuum Pans
Traps
Vapors
Vapors
Feed Water Heater
Exhaust Main
Exhaust Heaters
Live Steam Heaters
Eliminators
Vapors
Traps
Rel Valve 15"
Open Receiver
Mud Tank
Boiler Feed Receiver
Closed Receiver
To mills for maceration, Make up Feed Water etc
To Boiler

Fig. 4

the boiler feed receiver. As shown, the juice heaters, using exhaust steam, discharge their condensation by means of a trap to an open receiver. The pressure in the exhaust heaters is seldom much above atmosphere. The hot juice leaving the live steam juice heaters usually has a temperature of 220°. On its way to the Deming separators it is passed through eliminators consisting in the main of closed drums with practically atmospheric pressure in them. The object of these eliminators is to remove and make possible the utilization of the heat in the juice above 212°. This heat passes off in vapor, which mixes with that from the auxiliary pan receiver and which is utilized in heating the feed water or in pre-heating the juice entering one of the Lillie evaporators and the Kestner evaporator, also in heating scums in the mud tanks. At the time the tests were being made, the average temperature of the boiler feed water leaving the boiler feed receiver averaged about 227°.

As stated elsewhere, the evaporating plant consists of three Lillie quadruple effects and one Kestner quadruple effect, each with a rated capacity of 400,000 gallons of juice per day, also six vacuum pans of the coil type, four of which are 12 ′ in diameter and two of which are 12′ 6″ in diameter. The average distance traveled by the condensation from the vacuum pans, the evaporators, and the heaters to the boilers is 540 feet, 550 feet, and 430 feet, respectively. The juice from the mill is heated by four tubular heaters arranged in two sets. The first set is heated by exhaust steam and the second set by live steam, about 70% of the work being done by the exhaust heaters. Most of the pumps handling liquids in this factory are of the direct acting steam type. Salt water is used for condenser injection. It is first pumped to the factory by large centrifugal pumps located at the water's edge. It is then lifted by reciprocating pumping engines of the fly wheel type to a small central tank in the top of the factory, the total lift being approximately 70 feet. Each quadruple effect and each vacuum pan has its individual condenser of the parallel current type. The injection water is supplied to these condensers from an injection water main, through branch lines controlled by valves. These condensers are also connected to air mains served by a number of vacuum pumps, some of which are of the rotative dry vacuum type and some of the slow speed, water sealed type. This factory has no hoisting apparatus outside of the factory, the railroad switches being such as to allow the passage of cars through the mill room. This reduces the amount of hoisting to a minimum. All steam pipes and containers are thoroughly insulated with pipe covering. The same may be said of liquid lines and containers. In fact, the latter is unusually well cared for.

A list of the principal apparatus comprising the equipment of this factory will be found in Table 1, Appendix B.

PROCEDURE IN MAKING THE EXPERIMENTS.

The principal steam consuming stations at this factory are the three Lillie quadruple effects A, B, and C; the Kestner quadruple effect; the vacuum pan work, which may be subdivided into straight strikes, mixed strikes, and crystallizer strikes; the live steam juice heaters, the exhaust steam juice heaters, the Kestner juice heater, and steam power. The general procedure in carrying on the tests was much the same as that described for Adeline, namely, individual tests of steam consumption and thermal efficiency were made for the different stations and from these data, together with other general factory data from which the quantities handled at each station were found, the steam consumption for the different stations was calculated. In making the individual tests on the evaporators, vacuum pans, and heaters the condensation was weighed on scales. Other necessary observations of pressure, temperatures, etc., were also made. The factory is unusually well equipped with scales, Venturimeters, etc., for weighing or measuring cane, juice, etc. By means of such equipment, the factory is able to maintain very complete records of this quantity. The period covered was from the beginning of the season up to and including February 22, 1914, a period of about two months.

Results of the Tests.

Table 6 contains general factory data obtained from the laboratory which has been used in the calculations.

TABLE 6.

GENERAL FACTORY DATA USED IN CALCULATING EVAPORATOR AND VACUUM PAN TESTS.

(Guanica.)

Average for Period.

% Dilute juice on cane	107.5
Brix of dilute juice	13.34
% Syrup on cane	29.05
Brix of syrup	56.5
Weight of massecuite per strike, tons, average	49.4
Number of straight strikes	446
Number of mixed strikes	369
Number of crystallizer strikes	329
Cane ground, tons	261,702.6
SUGAR MADE, TONS	28,506.16

Table 7 contains other data used in the calculations which were obtained through the individual tests.

TABLE 7.

DATA FROM INDIVIDUAL TESTS.

(Guanica.)

Average, boiler pressure, lbs. gage	90
Quality of steam, % dry	98.5
Temperature boiler feed water	227.0
Factor of evaporation	1.009
Work done by Kestner evaporator, % of total	35.0
Work done by Lillie (A)	25.0
Work done by Lillie (B)	20.0
Work done by Lillie (C)	20.0
Water evaporated per lb. of steam, lbs.—	
Kestner evaporator	4.24
Lillie evaporator (A)	3.71
Lillie evaporator (B)	3.69
Average for all evaporators	3.93
Vacuum pans, straight strikes	.845
Vacuum pans, mixed strikes	.850
Vacuum pans, crystallizer strikes	.811
Average for all pan work	.835

It may be well also to call attention to the fact that additional data regarding these individual tests may be found in Louisiana Bulletin 149.

In order to determine the amount of steam consumed for power (mechanical energy) horse-power tests were made on most of the engines, pumps, and motors in the house. It was not possible, however, to do this very accurately, especially for the electric motors, on account of the large number of them and the difficulty of connecting up the electric measuring instruments. Table 8 gives a list of the electric motors with rated H. P., also developed H. P. where it was possible to obtain same.

TABLE 8.

H. P. OF MOTORS (Guanica).

	Rated H. P.	Developed H. P.
6 A Centrifugals	20	....
1 A Mixer	15	9.50
1 A Molasses pump	10	....
1 No. 2 centrifugal scroll conveyor	25	9.00
1 B Centrifugal	100	80.4
1 C Centrifugals	100	51.6
12 D Centrifugals	20	......
1 E Centrifugals	100	48.2
1 Old crystallizers	30	15
1 New crystallizer	50	15
1 Electric vacuum pump	35	30
1 No. 2 Cross conveyor (Servell)	20	16.6
1 No. 1 Cross conveyor, Sc	10	7.2
1 No. 1 washing machine	15	9.0
1 No. 2 washing machine	15	8.2
1 Kestner centrifugal feed pump	10	....
1 Hot juice pump	10	5.9
1 Deming eliminator pump	30	17.6
1 Murphy eliminator pump	30	17.6
1 A Quad. centrifugal pumps	100	71.5
1 B Quad. centrifugal pumps	75	45.0
1 C Quad. centrifugal pumps	75	31.8
1 Lime house	15	12
1 Main bagasse conveyor	15	....
1 Strainer tank	7.5	5.2
1 North end bagasse conveyor	7.5	....
1 South end bagasse conveyor	7.5	....
1 Cross bagasse conveyor	15	8.0
1 Climax bagasse conveyor	15	10.0
1 Main bagasse conveyor	35	18.0
1 Return bagasse conveyor	15	9
1 Magma pump	15	19
1 Disintegrator	5	....
1 No. 1 salt water pump	160	154
1 No. 2 salt water pump	160	154
8 Hoist cranes	25	....
4 Hoist cranes	3.5	....
2 Hoist sugar W. H	7.5	....
1 Hoist sugar W. H	3	....
Total	....	1359

In making up the total the "rated H. P." was used where the "developed H. P." was lacking.

Table 9 gives a summary of the power consumed for factory purposes, including the 1359 H. P. for motors.

TABLE 9.

Power Consumed for Factory Purposes.

(Guanica.)

	I. H. P.
Mill A, Crusher, first and second mills	265.32
Mill A, Third and fourth mills	220.00
Mill B, Crusher	52.17
Mill B, First mill	91.88
Mill B, Second mill	91.88
Mill C, Crusher	72.00
Mill C, First, second and third mills	366.16
Mill C, Fourth mill	139.42
Mill D, Crusher and first mill	177.94
Mill D, Second, third and fourth mills	330.02
Salt water pumps (cards)	85.90
Raw juice pumps (calculated from pressure)	40.00
Hot water pump	14.00
Maceration pump	6.50
Six engines, cane carrier	42.00
Vacuum pumps	318.00
Cane carrier drag engines	24.00
Motors	1359.00
Boiler feed pumps (5)	18.17
Mill juice pumps	20.00
Cachaza, Kestner S. water, Kestner syrup (crystallizer, Lillie syrup pumps and miscellaneous)	18.00
Total	3752.36

The steam consumed for power was calculated as follows:

Tons cane per 24 hours (basis)	4,300
Equivalent B. T. Us. per minute, corresponding to $3752 \text{ H. P.} = \frac{3752 \times 33{,}000}{778} =$	159,146
Equivalent lbs. water F. & A. 212° per minute, 159,146 ÷ 970.4	164
Tons cane ground per minute, 4300 ÷ (24 × 60)	3.0
Equivalent steam F. & A. 212° per ton cane, lbs	54.67
Equivalent steam F. & A. 212°, for power, % on cane	2.733

Table 10 gives a summary of steam consumed, etc., at the different stations. As heretofore stated, it was not possible to make tests of steam generated in boilers. Item 11, radiation and unaccounted for, has been assumed to be 12% of the weight of the cane. This is probably not far from the accurate figure. Item 12 also is based on this assumption.

TABLE 10.

Summary of Steam Consumption Tests.

(Guanica.)

	Condensate per Ton Cane, lbs.	Steam F. & A. 212° per Ton of Cane, lbs.	Equiv. lbs. Steam at Boiler, per Ton Cane	Steam F. & A. 212° % on Cane	Steam F. & A. 212° % of Total	Boiler H. P. per Ton Cane	Boiler H. P. 4500 Tons per 24 Hrs.
1. Evaporators	417.0	413.7	410.0	20.68	28.2	.4985	2242.3
2. Vacuum pans, straight.	191.4	192.7	191.0	9.63	13.17	.2323	1045.3
3. Vacuum pans, mixed...	164.2	164.87	163.4	8.24	11.02	.1887	849.15
4. Vacuum pan crystallizer	89.32	90.00	89.2	4.50	6.11	.1084	477.8
5. Vacuum pan average...	444.92	447.57	443.6	22.37	30.3	.5395	2427.7
6. Live steam heaters....	81.5	81.4	80.7	4.07	5.52	.0977	439.6
7. Exhaust steam heaters.	210.7	208.6	206.7	10.43	14.16	.2512	1130.4
8. Kestner heater........	28.0	27.75	27.5	1.39	1.88	.0332	149.2
9. Equivalent of mechanical work..........	......	54.66	54.17	2.73	3.72	.0657	295.65
10. Total accounted for in factory.............	1182.12	1233.68	1222.67	61.67	83.70	1.4859	6686.5
11. Radiation, and unaccounted, assumed...	......	239.0	236.8	12.00	16.22	.2790	1246.0
12. Total delivered by boilers †	......	1479.68	1459.47	73.67	100.00	1.7649	7932.5

† Based on the assumed radiation and unaccounted for of 12%.

Heat Losses From Hot Liquid Lines and Containers.

A series of temperature measurements were made in a manner similar to those at Adeline, the object of which was to get some information relative to heat losses from hot liquid lines and containers. The methods used in securing these data were similar to those described for Adeline. Table 11 gives the results obtained from these observations.

TABLE 11.

(Guanica.)

	Steam, Per Cent of Weight of Cane	Per Cent of Total (Approximate)
Radiation loss, hot water lines..........................	.84	1.14
Radiation loss, juice lines and tanks..........................	2.02	2.74
Radiation loss, syrup lines and tanks..........................	.082	.11
Radiation loss, molasses lines and tanks..........................	.085	.11
Total, hot liquid containers..........................	3.027	4.10

EXPERIMENTS AT FLORIDA.

OBJECT OF THE EXPERIMENTS.

As stated heretofore, Central Florida is driven entirely by electric power. In fact, this factory is said to be one of the first, if not the first, house planned from the beginning for all-electric drive. The object of the experiments was to obtain data that would bear upon the question of the relative merits of electric drive. This included not only power measurements but especially the determination of the ratio of exhaust steam to direct or boiler steam used. The present tendency in sugar house design is to reduce the amount of exhaust steam to a minimum, so as to make possible the use of vapor juice heaters, pre-evaporators, etc., for reducing the fuel consumption to a minimum.

FACTORY EQUIPMENT AND PROCESS.

This house was designed to grind 1000 tons of cane per 24 hours. The arrangement of the machinery is such that the house is very open, platforms being used instead of floors, with the result that one can stand at almost any place in the factory and see almost the entire lay-out. A list of the principal equipment of the house is given in Appendix C.

The cane is unloaded from the cars by means of an hydraulic side-dumping platform designed and patented by Cuervo & Pagliery. Each car of cane is dumped sidewise by releasing water from two hydraulic cylinders supporting one side of the track on which the car stands, the other side being hinged. A vertical triplex power pump, driven by an electric motor, supplies water to the two hydraulic cylinders. The cane falls upon a short, horizontal carrier, which delivers it to the main cane carrier. The mill is a 12 roller outfit. The generating station consists of two 800 Kw. steam turbines, direct connected to A. C. generators. One of the turbines is sufficient to carry the load, the other one being kept in reserve for emergencies.

The boiler plant consists of six H. R. T. boilers, each rated at 250 H. P. Four of these boilers are equipped with Foster superheaters, which are designed to deliver steam to the steam turbines at 125 pounds gauge and 100 degrees superheat. The other two boilers supply steam for heating and evaporating through a separate steam main. The furnaces are of a special Dutch oven type, the entire setting being encased in sheet steel. All carriers are operated by electric motors. The pumping in the factory is done by centrifugal pumps direct connected to electric motors. The molasses and filter press scums are handled by means of vertical, triplex power pumps, direct connected to electric motors. In most cases, individual motor drives are used, though there are a few groupings. For example, the washing machine for filter press cloths and centrifugal wringers, also the filter press mud drag, are driven by one motor through belts and shafting.

The evaporating plant consists of a quadruple effect of the standard type, with the first body 10 feet in diameter and the other three bodies 9 feet in diameter. Vapor is taken off from the first body for heating the cold juice from about 80 degrees to 190 degrees, the balance of the heating being done by exhaust or live steam. There are two 11 foot vacuum pans with double calandrias of the express type. These two vacuum pans are connected to a single vapor pipe served by a single No. 11 Westinghouse-LeBlanc low-level condenser situated on the ground floor. There is also an auxiliary vacuum pump of the Rotrex type used for raising vacuum quickly in the pans. The last body of the quadruple effect is also served by another No. 11 Westinghouse-LeBlanc low-level condenser. Both of the low-level condensers discharge their hot injection water into a main, which delivers it to a spray pond outside of the factory against a pressure of 10 to 12 pounds per square inch. The vacuum in the condenser draws the injection water back from the spray pond. The centrifugal pumps are all of the single stage type manufactured by the Allis-Chalmers Company. The juice as it leaves the mill is pumped to juice scales situated at practically the highest point in the factory. From this point it flows, with a few exceptions, by gravity through the various steps in the process. The steam pipes and containers through the house are well insulated with 85% magnesia covering. The vacuum pans operate with steam pressures of 7 pounds gauge or less, the same being true of the juice heaters. The temperature of all the condensation fit to use for boiler feed is, therefore, of relatively low temperature. This being the case, there would be little saved by returning it to the boilers under pressure. Consequently, the system for handling condensation is of the open type illustrated by Figure 5. The only trap used in handling condensation is in connection with the vacuum pan. The juice prior to clarification is heated

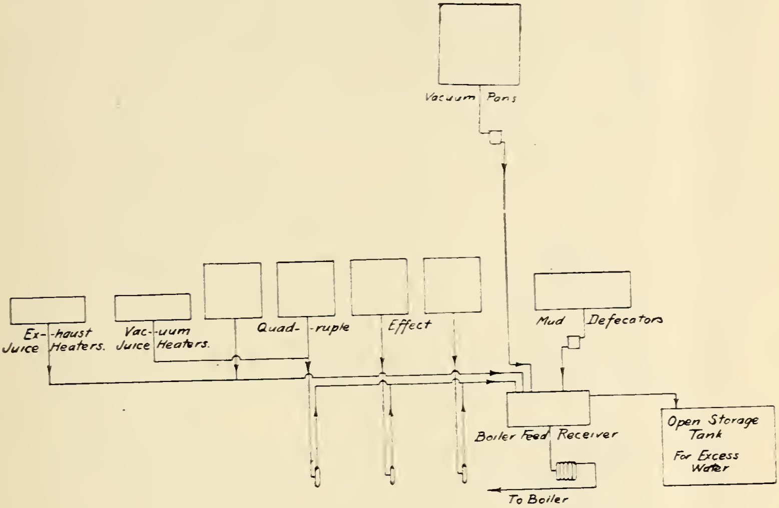


Fig. 5

to a temperature of 210 degrees or lower; consequently, there is no loss of heat by flash. The water supply (make-up) for the factory is pumped from a spring approximately 1000 feet from the factory to a tank in the factory about 70 feet above the floor.

The product of the factory is 96 test raw sugar and low grade molasses. Figure 6 shows the juice flow and the method of boiling.

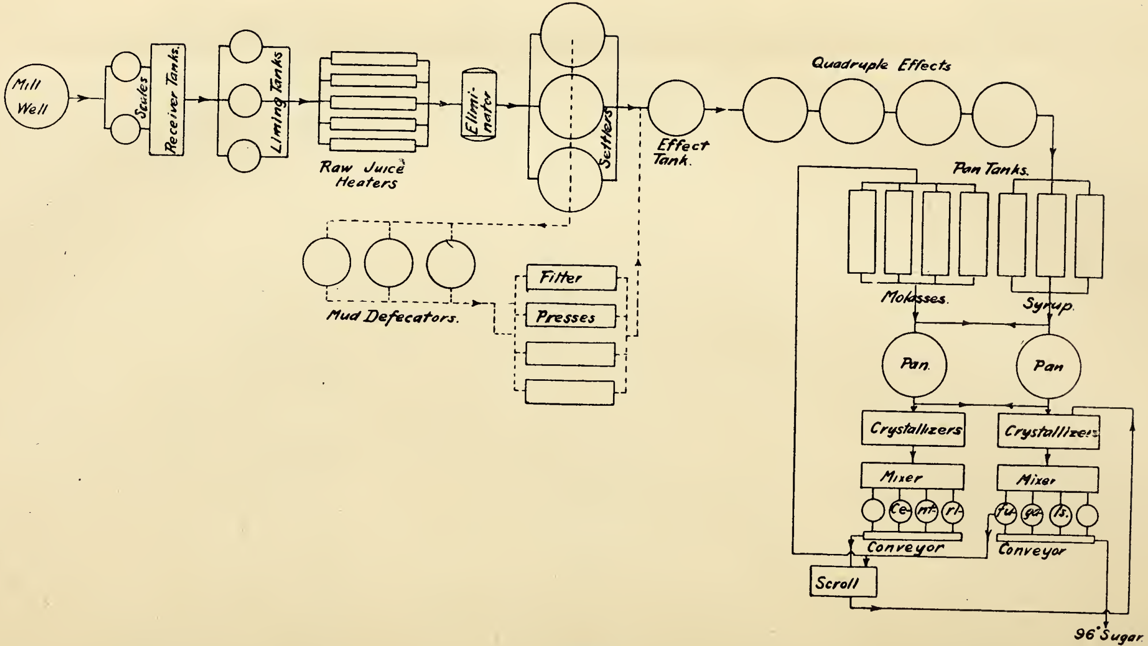

Mill Well
Scales
Receiver Tanks.
Liming Tanks
Raw Juice Heaters
Ellimi-nator
Setters
Mud Defecators.
Filter
Presses
Effect Tank.
Quadruple Effects
Pan Tanks.
Molasses.
Syrup.
Pan.
Pan
Crystallizers
Crystallizers
Mixer
Mixer
Ce-
nt-
ri-
fu-
ga-
ls.
Conveyor
Conveyor
Scroll
96° Sugar.

Fig. 6

PROCEDURE IN MAKING THE EXPERIMENTS.

The data obtained in these experiments, as will be seen, differ somewhat from those at Adeline and Guanica in that no steam consumption tests were made at individual stations. Practically all of the exhaust steam in the factory comes from the steam turbine of the generating station. This exhaust steam is consumed in the quadruple effect, the vacuum pans and the exhaust steam juice heaters, the deficiency at these stations being made up with direct (boiler) steam, all of which is generated in the two house boilers. The larger part of the boiler steam goes to the vacuum pans. Pressure reducing valves are used to reduce the pressure for use at these stations. Boiler steam is also used in the scum blow-ups. The steam consumption tests were confined entirely to the measurement, during a record run, of the steam simultaneously supplied to the steam turbine and direct steam for the house. This was done by simultaneous measurements of the feed water to the two sets of boilers, indicating Venturimeters being used. It was planned to have this test last 24 hours, but, due to a shut-down of the house, it was necessary to limit it to 15 hours.

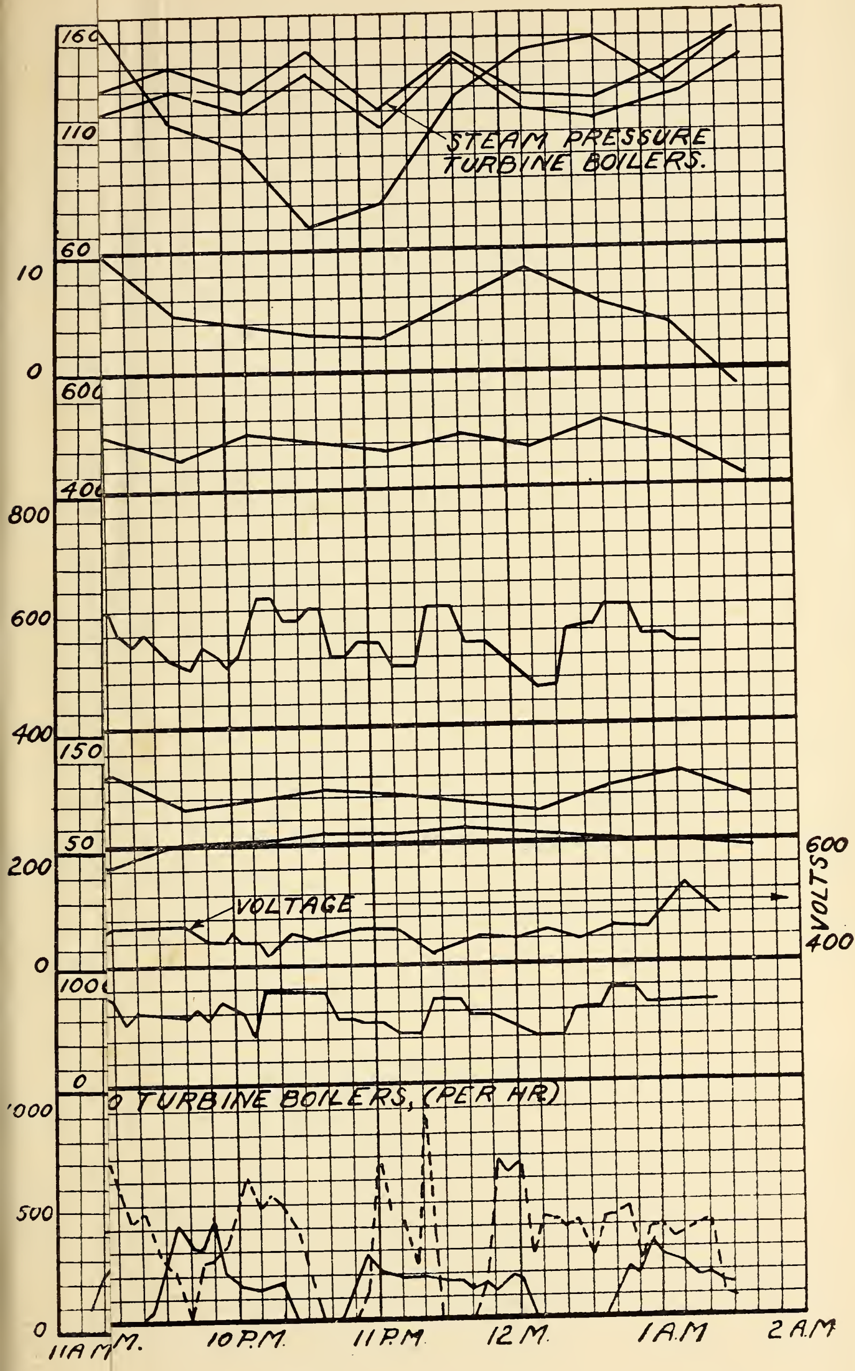
Power measurements of most of the motors in the house were also made. For this purpose a Westinghouse portable indicating Wattmeter was used, together with the necessary voltage and current transformers. The instrument was a good one, but unfortunately it was not possible to calibrate it. In view of this, we cannot be definitely sure that these measurements are absolutely accurate, though it is probable that they are.

In the case of the larger motors, and especially those with variable load, the power observations were made at frequent intervals throughout a considerable period of time. This is especially true of the motors operating the mills, the condensers, etc. In the case of small motors with little load variation only a few readings were taken.

Results of the Experiments.

Table 12 gives the principal results obtained from the tests on the boilers supplying steam to the steam turbine, also the boilers supplying direct steam to the house. The Venturimeter affords a very accurate means for measuring water, especially under the conditions obtaining in these particular tests, namely, with the centrifugal feed water pump, which gives a very steady flow. However, especially in the case of the turbine boilers, the flow through the meter was considerably below its normal capacity. Under this condition, the Venturimeter is less accurate than when operating at normal capacity. However, the error, if any, should not exceed three or four percent. The Venturimeter used with the house boilers operated near its rated load and should be accurate to within one percent. During the tests the safety valves on the house boilers blew off occasionally, which introduced a small unmeasurable error. This error, however, is so small as to be negligible.

Figure 7 is a graphical log of the principal observations made in these tests, together with some calculated values.


16
110
STEAM PRESSURE
TURBINE BOILERS.
60
10
0
60
40
800
600
400
150
50
200
600
VOLTS
400
VOLTAGE
0
100
0
000
0 TURBINE BOILERS, (PER HR)
500
0
11AM
M.
10P.M.
11P.M
12M.
1A.M
2A.M

Results of the Experiments.

Table 12 gives the principal results obtained from the tests on the boilers supplying steam to the steam turbine, also the boilers supplying direct steam to the house. The Venturimeter affords a very accurate means for measuring water, especially under the conditions obtaining in these particular tests, namely, with the centrifugal feed water pump, which gives a very steady flow. However, especially in the case of the turbine boilers, the flow through the meter was considerably below its normal capacity. Under this condition, the Venturimeter is less accurate than when operating at normal capacity. However, the error, if any, should not exceed three or four percent. The Venturimeter used with the house boilers operated near its rated load and should be accurate to within one percent. During the tests the safety valves on the house boilers blew off occasionally, which introduced a small unmeasurable error. This error, however, is so small as to be negligible.

Figure 7 is a graphical log of the principal observations made in these tests, together with some calculated values.

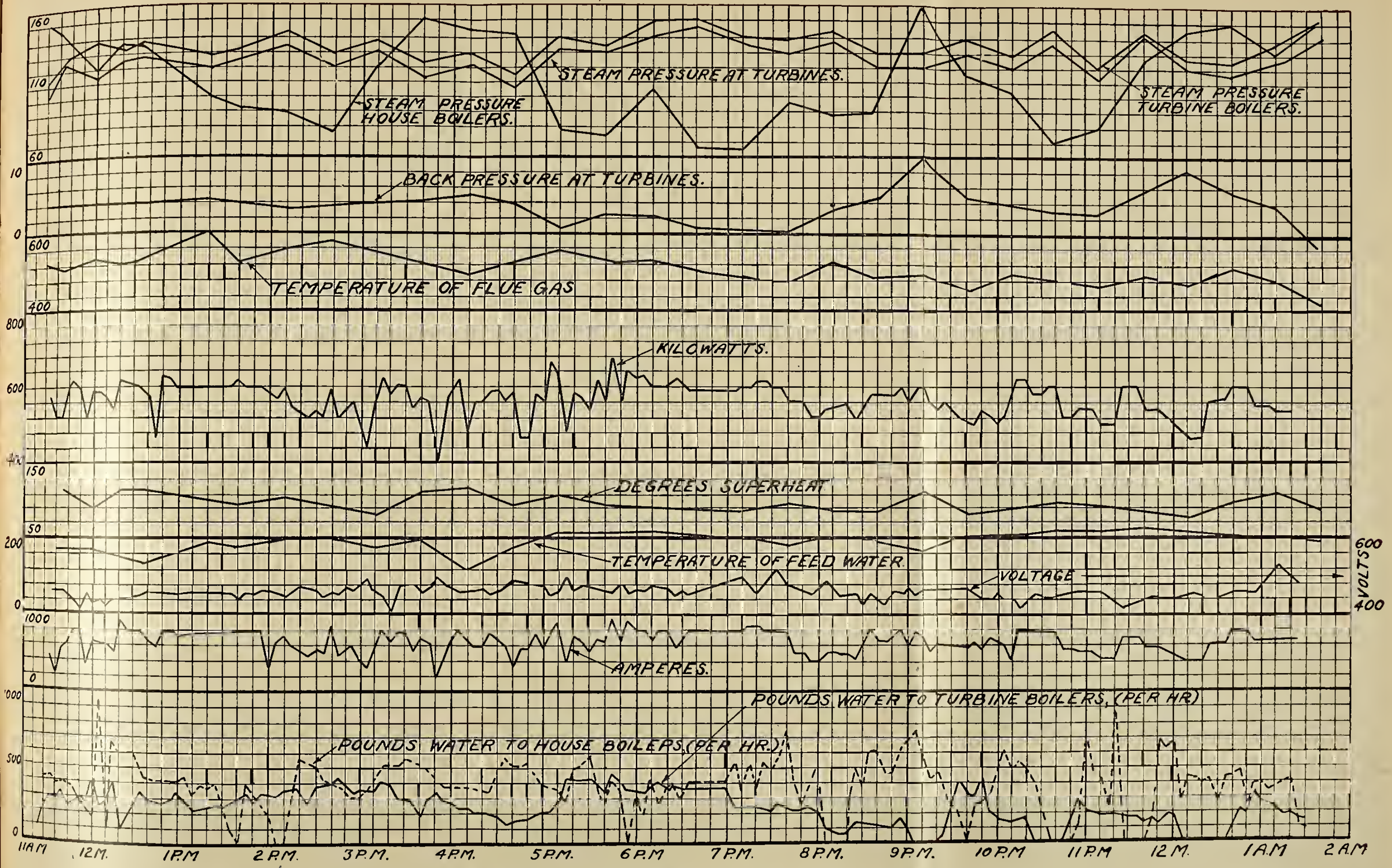

160
110
STEAM PRESSURE AT TURBINES.
STEAM PRESSURE HOUSE BOILERS.
STEAM PRESSURE TURBINE BOILERS.
60
10
BACK PRESSURE AT TURBINES.
0
600
TEMPERATURE OF FLUE GAS
400
800
KILOWATTS.
600
150
DEGREES SUPERHEAT
50
200
TEMPERATURE OF FEED WATER.
VOLTAGE
600
VOLTS
400
0
1000
AMPERES.
0
1000
POUNDS WATER TO TURBINE BOILERS, (PER HR)
POUNDS WATER TO HOUSE BOILERS (PER HR.)
500
0
11 A.M.
12 M.
1 P.M.
2 P.M.
3 P.M.
4 P.M.
5 P.M.
6 P.M.
7 P.M.
8 P.M.
9 P.M.
10 P.M.
11 P.M.
12 M.
1 A.M.
2 A.M.

Fig. 7

TABLE 12.

Test of Boiler Plant and Steam Turbine.

(Central Florida.)

1.	Date of Test	Jan. 19, 1916
2.	Duration of test, hours	14.35
3.	Rate of grinding (36.4 tons per hour), average tons per 24 hrs.	873.6
4.	Extraction, %	81.6
5.	Moisture in bagasse, approximate, %	49.4
6.	Temperature feed water	195.3
7.	Temperature flue gases	510
8.	Atmospheric pressure, lbs. per sq. in.	14.70
9.	Reading at switch board, average K. W.	577

Turbine Boilers.

10.	Steam pressure turbine boilers, lbs. gage	133.6
11.	Degrees superheat of steam to turbine boilers, °F	87.8
12.	Steam pressure at turbine, lbs. gage	125.2
13.	Back pressure on turbine, lbs., gage	3.56
14.	Water fed to turbine boilers per hour	22,248.36
15.	Steam per K. W. hr., lbs	38.55
16.	Steam per H. P. hour, at switchboard, lbs	28.76
17.	Boiler H. P. developed by turbine boilers	717.78

House Boilers.

18.	Steam pressure house boilers, lbs. gage	110.9
19.	Water fed to house boilers per hour, lbs	13,000
20.	Quality of steam	99
21.	Factor of evaporation	1.049
22.	Water evaporated F. & A. 212° per hour, lbs	13,620
23.	Boiler H. P. developed by house boilers	397.5
24.	Total boiler H. P. of house	1,115.3
25.	B. H. P. per ton of cane per 24 hours	1.277
26.	Exhaust steam from turbines, % weight of cane	30.52
27.	Direct steam for house, % weight of cane	14.85
28.	Total steam, % weight of cane	45.37

Power Tests.

(Florida.)

Table 13 gives a summary of the electrical power tests made at Florida. The methods and apparatus used in making these tests have already been described on page 33.

Summary.

TABLE 13.

Power of Electric Driven Apparatus.

(Florida.)

		E. H. P. Developed by Motor	Rated H. P. of Motor
1.	Crusher, first and second mills (33 tons per hour)	(194.0)	250
2.	Third and fourth mills (29.4 tons per hour)	(162.2)	200
3.	Third and fourth mills (36.5 tons per hour)	(186.2)	200
4.	Third and fourth mills (38.25 tons per hour)	(194.0)	250
5.	Crusher, first and second mills (42.6 tons per hour)	191.5	250
6.	Crusher, first and second mills (53.4 tons per hour)	210.0	250
7.	Centrifugals (both stes)	83.2	......
8.	Westinghouse-LeBlanc condenser (pan)	87.4	80.0
9.	Westinghouse-LeBlanc condenser (quad)	88.2	80.0
10.	Crystallizers	......	20.0
11.	Bagasse carrier to storage and boilers	......	20.0
12.	Cane carrier	......	15.0
13.	Electric hoist for unloading wagon	......	15.0
14.	Sugar elevator and screw conveyor	6.2	10.0
15.	Cush-Cush elevator	2.0	10.0
16.	Bagasse drag	2.8	7.5
17.	Bagasse hoppers	0.6	3.0
18.	Filter press mud drag, washing machine and dryer, carrier only	2.8	10
19.	Carrier and washing machine—latter empty	3.1	10
20.	Carrier and dryer only—latter empty	3.8	10
21.	Auxiliary cane carrier	......	3
22.	Hydraulic cane dump (pump)	3	......
23.	Juice heater pump	20.6	25.0
24.	Rotrex vacuum pump	10.7	10.0
25.	Syrup pump	3.9	5.0
26.	Sweet water pump second body, also for condensation from vapor juice heaters	1.6	5
27.	Quad. juice feed pump	2.2	3.0
28.	Triplex mud pump	2.4	3.0
29.	Molasses pump for first centrifugals	1.9	3.0
30.	Molasses pump, second centrifugals	......	3.0
31.	Mill journal and maceration water pump	1.2	2.0
32.	Mill well pump, first stage	6.4	7.5
33.	Mill well pump, second stage	7.4	7.5
34.	Lime mixers and lime pumps	3.6	3.0
35.	Magma pump	......	10.0
36.	Centrifugal feed water pump, fifth stage	......	50.0
37.	Pump from boiler recipient to house hot water tank	......	10.0
38.	Centrifugal pump (water supply) at spring	......	20.0

In the tests of the Westinghouse-LeBlanc condenser, the motor horse-power of which is given in Table 13, other observations bearing on the result were made. These are included in Table 14.

The weight of vapors condensed during the test was determined by means of a simultaneous test on the quadruple effect from which the evaporation and the weight of vapor leaving the last body and entering the condenser were calculated. The various observations were made every 20 minutes.

TABLE 14.

TEST OF WESTINGHOUSE-LEBLANC CONDENSER

(Quadruple Effect.)

Date of test	Jan. 18, 1916
Duration of test, hours	4.48
Temperature of injection water entering	85.9
Temperature of injection water leaving	102.2
Vacuum in condenser, inches mercury	24.8
Vacuum at air pump inlet, inches mercury	6.2
Barometer, inches mercury	29.90
Temperature of room	86.2
Temperature outside	82.7
Pressure in discharge pipe of ejection pump, lbs. gage	12.7
Power developed by motor (from Wattmeter reading) H. P.	88.2
Vapors to condenser during test, lbs	44,848
Injection water per lb. of vapor, lbs	60.39
Total injection water used, lbs	2,708,505
Injection water per hour, lbs	604,128

TEST OF SPRAY POND.

While making power tests of the Westinghouse-LeBlanc low-level condenser which serves the vacuum pans, additional observations were made bearing not only upon the performance of this condenser but the spray pond as well. Although these observations do not bear directly upon the subject of power consumed, they will, perhaps, be of interest to some, for which reason they are submitted. These observations will be found in Table 15. In determining the amount of cooling done by the spray pond, it was necessary to ascertain the amount of injection water it handled, also the drop of temperature in the pond. Since the condensers serving both the vacuum pans and the quadruple effect were discharging hot injection water into the spray pond, it was necessary to determine the weight of vapors being discharged by the vacuum pans and the last body of the quadruple effect into the condensers. With these quantities and the initial and final temperatures of the injection water at the condensers known, it was possible to calculate the weight of injection water. It should be noted that the weight of injection water determined in this manner is somewhat approximate, as it was impossible to obtain very accurate measurements of so many different quantities simultaneously. The results, however, will be of some value in a general way.

TABLE 15.

Spray Pond Test.

(Florida.)

1.	Date of test	Jan. 8, 1916
2.	Duration of test, hours	7.1
3.	Barometer reading, inches mercury	29.87
4.	Temperature of room	76.5
5.	Temperature outside	73.0
6.	Relative humidity	62.9
7.	Temperature injection water entering pan condenser	86.7
8.	Temperature injection water leaving pan condenser	99.0
9.	Vacuum in pan condenser, inches mercury	26.40
10.	Vacuum at air pump inlet, pan condenser, inches of mercury	6.80
11.	Vapor to pan condenser during test, lbs	67,784
12.	Injection water, per lb. of vapor, pan condenser, lbs	79.8
13.	Injection water, pan condenser, lbs	5,409,163
14.	Injection water per hour, pan condenser, lbs	761,854
15.	Pressure on discharge of cooling water pump, pan condenser, lbs. gage	10.6
16.	Power developed by motor of pan condenser (from Wattmeter readings) H. P.	86.7
17.	Temperature injection water entering quad condenser	88.2
18.	Temperature injection water leaving quad condenser	107.5
19.	Vacuum in quad condenser, inches mercury	25.4
20.	Vapor to quad condenser, lbs	112,328
21.	Injection water, per lb. of vapor, quad condenser, lbs	50.62
22.	Total injection water used, quad condenser, lbs	5,686,043
23.	Injection water per hour, quad condenser, lbs	800,851
24.	System, Spraco.	
25.	Number of spray nozzles	100
26.	Cooling effect per hour, pan injection, B. T. U., (99.0 — 86.7) 761,854 =	9,380,801
27.	Cooling effect per hour, quad injection, B. T. U., (107.5 — 88.2) 800,851 =	15.456,424
28.	Cooling, total, B. T. U. hour	24,837,225

GENERAL SUMMARY.

Table 16 includes some of the most general data secured in these tests that will facilitate comparisons of the steam power consumption at the three factories. As heretofore stated, the power measurements at Guanica were somewhat crude, and for that reason too much dependence should not be placed in them. It should also be remembered that the data at Guanica for boiler feed water—that is, steam generated—is based on an assumed radiation and unaccounted for in the factory of 12% on the weight of cane ground. Guanica has the advantage in being a very large factory, for it goes without saying that both power and heat losses are proportionately smaller in a large factory than in a small one. In comparing the steam consumption at Adeline and Guanica it will be noted that the steam for the vacuum pans was very much less at Adeline. This is due to the fact that the pan work at Adeline requires very much less boiling back. This advantage, however, was partly offset by the increased amount of re-heating of juice and syrup, also granulation, at Adeline. The weight of exhaust steam at Florida was determined accurately, though that at Adeline is approximate, having been estimated from the I. H. P. tests of the different engines and pumps given in Table 2, page 15. The water rates assumed in this calculation were 30 pounds H. P. hour for Corliss engines, 125 pounds H. P. hour for direct acting steam pumps, and 60 pounds H. P. hour for the bagasse drag engine. The water rate of 30 seems rather high for Corliss engines, though in view of the general underloaded condition, it is probably not too high.

TABLE 16.

	ADELINE	GUANICA	FLORIDA
PRODUCT	White Granulated Sugar. Saleable Molasses	96 Test Sugar. Low Molasses.	96 Test Sugar. Low Molasses.
SYSTEM OF EVAPORATING	Plain Quad.	Plain Quad.	Quad. and Vapor Juice Heater.
POWER SYSTEM	Centrifugal Pumps, Belt and Shafting	Part Electric Motor Drive. Part Direct Steam Drive	All Electric Motor Drive.
Power per ton cane per 24 hours, H. P.	.65	.873	.98
Steam for power, % cane	2.06	2.73	3.02
Steam for pans, % cane	14.48	22.37	....
Boiler feed water F. & A., % cane	71.95	73.67 †	45.37
Radiation and unaccounted F. & A., % cane	13.94	12.0 †	....
Exhaust steam, % cane	28.8	....	30.52
Ratio exhaust steam to total steam	.40	....	.67
Rated capacity, tons per 24 hours	1500	4500	1000
Capacity, time of tests	1380	4300	875

†Based on the assumed radiation and unaccounted of 12%.

APPENDIX A.

EQUIPMENT, ADELINE SUGAR FACTORY.

6 H. R. T. boilers, 84" x 20', 2500 sq. ft. H. S. each, for bagasse.
2 H. R. T. boilers, 84" x 20', 2500 sq. ft. H. S. each, for oil.
1 H. R. T. boiler, 150 H. P.
1 Heine boiler, 2500 sq. ft. H. S. each, for oil.
1 Twelve-roller mill and 1 Krajewski crusher preceded by a revolving cutter. Mill rollers 34" x 84", crusher rollers, 32" x 84".
2 Juice scales with tanks, capacity 10,000 lbs. each.
5 Liming tanks, 9' diam. x 11', 700 cu. ft. capacity each.
3 Raw juice heaters, 760 sq. ft. H. S. each.
    96 tubes each, 17' 4" long x 1¾" O. D.—16 passes, 585 sq. ft. H. S.
2 Thin juice heaters.
    96 tubes each, 11' 4" long x 1¾" O. D.—16 passes, 490 sq. ft. H. S.
2 Thick juice heaters,
    56 tubes each, 10' 10" long x 1¾" O. D.—28 passes, 275 sq. ft. H. S.
1 Molasses heater,
    56 tubes each, 6' 10" long x 1¾" O. D.—56 passes, 175 sq. ft. H. S.
    The tubes of all heaters are of brass and are No. 15 U. S. standard gauge, about 1/16" thick.
3 Deming separators.
6 Mud defecators with steam coils.
8 Kelly filter presses.
8 Bag filters, each 11' x 5' — ¾" x 6' high with 250 bags.
    Five filters used for the thin juice and three for thick juice.
    Danek filters for thin juice and for thick juice.
2 Swenson quadruple effects with 8000 sq. ft. H. S. each.
    Tubes 3½' long x ¾" O. D.—2000 sq. ft. per body.
2 Sugar melters with revolving stirrers.
2 Molasses melters with revolving stirrers.
3 Cylindrical syrup blow-up tanks 7' diam. with a capacity of 200 cu. ft. each.
Pan Charge Tanks. (For syrup, for molasses, and for wash.)
2 Express vacuum pans with single calandria, each 13' diameter and 750 sq. ft. H. S.
2 Mixers, one having a capacity of 371.2 cu. ft. and other having a capacity of 984.4 cu. ft.
    (Actually one large mixer with a cap. of 1355 cu. ft. divided into 2 compartments.)
8 Belt-driven Weston centrifugals, 40".
2 Hersey graulators working in tandem.
1 Crusher engine (Corliss), 16" x 42".
1 Engine driving first, second and third mills (Corliss), 32" x 60".
1 Engine driving fourth mill (Corliss), 24" x 48".
1 Bagasse drag engine (plain slide valve), 11" x 12".
3 Hoisting engines (derricks).
1 Hoisting engine (Walsh rake).
1 Electric light engine (automatic high speed), 12" x 12".
1 Engine driving centrifugals and pumps (Corliss), 26" x 48".
    This engine drives eight 40" Weston centrifugals and the following centrifugal pumps, through shafting and belts:
1 Thin juice pump.................................. 3" 1156 R. P. M.

1	Filter press pump	3″	1100	“
1	Sweet water pump	3″	1140	“
1	Thick juice pump	2″	1350	“
1	Water to presses pump	2″	1430	“
2	Evaporator drain pumps, second and third body	2″	1200	“
2	Evaporator drain pumps, fourth body	1½″	1000	“
2	Evaporator syrup pumps	2″	1035	“
1	Milk of lime pump	1½″	989	“
1	Soda to evaporators and pans	4″	780	“
1	Mill juice pump	3″	1100	“
1	Hot water pump	2½″	1223	“
1	Kelly press pump	2″	1170	“
1	Acid press pump	2″	925	“
1	Maceration water pump	2″	1375	“
2	Cold water pumps	8″	840	“
1	First body evaporator drain	2″	1000	“
1	Vacuum pump (vacuum cylinder 36″ x 24″), steam cylinder	22″ x 24″		
1	Raw juice pump, duplex system	14 x 10 x 12		
1	Mill well pump, single steam	21 x 14¾ x 24		
2	Boiler feed pumps, duplex steam	12 x 7 x 12		
2	Boiler feed pumps, drip, duplex, steam	12 x 8 x 12		
1	Thick juice pump to bag filters	12 x 7 x 12		

APPENDIX B.

EQUIPMENT, PARTIAL, CENTRAL GUANICA.

4 Climax bagasse boilers, 508 H. P. each.
2 Stirling bagasse boilers, 650 H. P. each.
2 Stirling bagasse boilers, 500 H. P. each.
2 Stirling oil boilers, 650 H. P. each.
3 Twelve-roller mills,
    A, mill rollers 34" x 78", crusher rollers 32" x 78".
    B, mill rollers 34" x 84", crusher rollers 28" x 84".
    C, mill rollers 34" x 78", crusher rollers 32" x 78".
1 Six-roller mill,
    D, mill rollers 32" x 72", crusher rollers 25" x 72".
8 Juice scales, with two tanks, 10,000 lbs. capacity each.
2 Deming juice heaters, 850 sq. ft. H. S. each.
2 Murphy juice heaters, 1005 sq. ft. H. S. each.
1 Juice heater (for Kestner), 618 sq. ft. H. S.
1 Juice heater (for Lillie (C)), 618 sq. ft. H. S.
5 Deming closed settlers.
3 Lillie quadruple effects, 10,000 sq. ft. H. S. each.
1 Kestner quadruple effect, 16,000 sq. ft. H. S.
4 12' Vacuum pans, triple coils, 1050 sq. ft. H. S. each.
2 12'-6" Vacuum pans, triple coils.
32 Crystallizers, capacity of about 50,000 lbs. of massecuite each.
33 40" Centrifugals.
Mill A, Engine driving crusher, first and second mills, Corliss.......28" x 54"
Mill A, Engine driving third and fourth mills, Corliss..............28" x 54"
Mill B, Engine driving crusher, Corliss...........................16" x 36"
Mill B, Engine driving first, second and third mills, Corliss..........34" x 60"
Mill B, Engine driving fourth mill, Corliss.......................24" x 54"
Mill C, Engine driving crusher and first mill, Corliss................26" x 48"
Mill C, Engine driving second, third and fourth mills, Corliss.......30" x 60"
Mill D, Engine driving crusher, Corliss...........................16" x 36"
Mill D, Engine driving first mill, Corliss.........................22" x 48"
Mill D, Engine driving second mill, Corliss.......................22" x 48"
1 Salt water pump, 18" x 14¾" x 30".
1 Raw juice pump, — x 12" x 30".
1 Hot water pump, 12" x 12" x 20".
1 Maceration pump.
6 Cane carrier engines.
Vacuum pumps.
4 Cane car drag engines.

APPENDIX C.

EQUIPMENT, CENTRAL FLORIDA.

4 H. R. T. boilers, 84″ x 20′, 2500 sq. ft. H. S. each, burn bagasse and wood, to supply turbines.
2 H. R. T. boilers, 84″ x 20′, 2500 sq. ft. H. S. each, burn bagasse and wood, to supply boiling house.
2 Allis-Chalmers steam turbines, 800 K. W. each, direct connected to Westinghouse A. C. generators. Phase 3, frequency 60, voltage 440, R. P. M. 3600.
Note.—Normally only one of these units is in service.
1 Twelve-roller mill and 1 Krajewski crusher.
Mill rollers are 32″ x 72″, crusher rollers 28″ x 72″.
2 Juice scales, with tanks, 10,000 lbs. capacity each.
3 Liming tanks of 7′ ¾″ dia. x 4′ 1″, 160 cu. ft. cap. each.
1 Heater charge tank, 8′ x 5′ x 1½″ x 4′, 164 cu. ft. cap.
3 Vapor raw juice heaters, each 225 sq. ft. H. S., 30 tubes, 15′ long x 2″ O. D.
2 Exhaust steam raw juice heaters, same as above.
8 Settling tanks, 10′ 1″ dia. x 5′ 7½″, 456 cu. ft. cap. each.
1 Receiving tank for clear juice. Two compartments, 198 cu. ft. total cap.
6 Mud defecators with steam coils, 6′ dia. x 3½′, 107.5 cu. ft. cap. each.
1 Filter press charge tank. Two compartments, 406 cu. ft. total cap.
6 Filter presses.
1 Effect charge tank, 7′ dia. x 4′, 154 cu. ft. cap.
1 Quadruple effect, 13,000 sq. ft. H. S.
4000 sq. ft. in first body and 3000 sq. ft. in each of the other bodies.
3 Pan charge tanks (syrup), 18′ x 8′ x 7½′, 720 cu. ft. cap. each.
4 Molasses pan charge tanks (molasses), same as above.
2 11′ double calandria express pans, 1208 sq. ft. H. S. each.
3 Crystallizers for first products, 7½′ dia. x 24′, 1053 cu. ft. cap. each.
6 Crystallizers for low grade products, 7½′ dia. x 20′, 879 cu. ft. cap. each.
1 Small mixer for first products.
1 Small mixer for low grade products.
5 Electric driven 42″ centrifugals for first products.
4 Electric driven 48″ centrifugals for low grade products.
2 Final molasses tanks.

MOTORS.

		H. P. of Motor	R. P. M.
1	Crusher, first and second mills	250.0	580
1	Third and fourth mills	200.0	580
1	Cush-cush elevator	10.0	860
1	Cane carrier	15.0	variable speed
1	Auxiliary cane carrier	3.0	variable speed
1	Hoist for unloading wagons	15.0	830
1	Bagasse drag	8.5	....
1	Bagasse hopper	3.0	850
1	Bagasse carrier to storage and boilers	20.0	1150
1	Filter press mud drag, washing machine and dryer	10.0	860
1	Lime mixer and small lime pumps	2.0	850
5	Centrifugals, first product	......	....
4	Centrifugals, low grade product	......	....
1	Crystallizer	20.0	860
1	Sugar elevator and screw conveyor	10.0	....

PUMPS AND MOTORS.

		H. P.	R. P. M.	Gals. per Minute
1	Mill well, first stage	7.5	1730	300
1	Mill well, second stage	7.5	1730	300
1	Mill journal and maceration water	2.0	1130	50
2	Juice heaters (2 units)	25.0	1750	300
1	Quad. juice feed	3.0	1140	300
1	Syrup, last body	5.0	......	....
1	Magma	10.0	870	8" x 12"
1	Molasses, first product centrifugals	3.0	850	40
1	Molasses, low grade product centrifugals	3.0	850	40
2	Triplex mud	3.0	570	....
1	Sweet water, last body	2.0	1130	100
1	Sweet water, third body	2.0	1130	100
1	Sweet water, second body	5.0	1150	200
1	Westinghouse-LeBlanc condenser, pans	80.0	700	....
1	Westinghouse-LeBlanc condenser, quad	80.0	700	....
1	Rotrex vacuum, pans	10.0	350	....
1	Hydraulic cane dump	3.0	......	....
1	Boiler feed, 5 stage	50.0	1750	220
1	Pump from boiler recipient to house hot water tank	10.0	1730	300
1	Water supply pump (at spring)	20.0	......	....

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EXPERIMENTAL STUDIES
— OF —
VACUUM JUICE HEATERS

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EXPERIMENTAL STUDIES OF VACUUM JUICE HEATERS.

OBJECT OF EXPERIMENTS.

The object of the experiments was to obtain data regarding the operation of vacuum juice heaters, including the effect of air in steam, juice velocity, absolute pressure of the heating steam, tube materials, and the length of vapor path upon heat transmission.

PRELIMINARY STATEMENT.

Practice shows that for the heating of juice that is necessary in clarification an amount of steam equivalent to 10% to 20% of the weight of cane ground is required for a 1000 tons house: this means 270 to 540 B. H. P. for heating. The variation in steam consumed is affected by the amount of maceration water used, the amount of juice in the cane, the amount of reheating done, the temperature range of the juice, etc. Most commonly this heating is done with exhaust or live steam in tubular heaters of the closed type.

The amount of water to be evaporated in the effects varies with conditions, but an average is 72% of the weight of the cane. Assuming that one pound of steam supplied to the first body will evaporate one pound of water from the juice in each body, the steam consumed for evaporation will be as follows:

Quadruple effect	$\frac{72}{4}$	18% on cane
Triple effect		24% “ “
Double effect		36% “ “

These values correspond to the following B. H. P. for a 1000 tons house:

Quadruple effect	500 B. H. P.
Triple effect	675 “ “ “
Double effect	1000 “ “ “

Assuming the average weight of steam for heating equal to 16% on cane, the total steam for heating and evaporating would be as follows:

Quadruple effect	18+16	34% on cane
Triple effect		40% " "
Double effect		52% " "

The above figures are, of course, based upon the heating of the juice with direct steam; that is, either exhaust or live steam. It is possible, however, to use vapor from the first or other bodies of the multiple evaporator for heating juice. By so doing, the total steam required for heating and evaporating may be made less than the figures given above. Since the boiling, even in the first body, usually takes place at pressures less than atmospheric, the pressure in such heaters is less than atmospheric, hence the term "vacuum heaters." In order to illustrate the use of vacuum heaters for juice heating, let us consider the following example.

Assume a quadruple effect with boiling temperatures as follows:

First body	216°
Second body	200°
Third body	178°
Fourth body	140°

Assume that the total evaporation is 72% on cane and that the steam or vapor required for heating is 16% on cane.

CASE 1.

Let the arrangement be as shown in Figure 1; namely, do all the heating of the juice—that is, 16%—with vapors from the first body. The temperature of the vapors formed in the first body as shown is 216°, which is high enough to raise the temper-

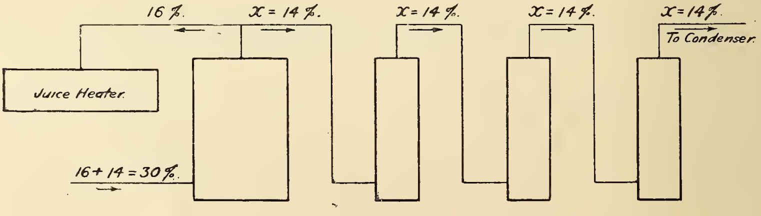

FIG. 1.

ature of the juice to, say, 212°. With a plain quadruple effect, the evaporation is practically the same in each body; that is, for our conditions 72÷4=18% on cane.

With the arrangement shown in Figure 1, the evaporation in the last three bodies will be practically the same, but a larger amount must be evaporated in the first body. In other words, sufficient steam must be liberated from the first body to supply the juice heater as well as the second body of the evaporator.

Let X = the percent evaporation in the last body.

Then X% will be evaporated in both the third body and the second body, while that in the first body must equal (X+16)%.

Total evaporated = 4X+16=72%

X=. 14%

Here, then, we have a total evaporation in the first body of 16+14=30% on cane, which will require a steam supply to the first body of 30%. In other words, the total for heating and evaporating with this arrangement is 30% on cane. As stated above, 34% would be required with a plain quad.

CASE 2.

In some cases, more than one vacuum heater may be used for heating juice. Figure 2 shows such an arrangement, the juice being first passed through a heater supplied with vapor from the third body and then through another heater with vapor from the first body. The temperature of the vapor from the third body being only 178°, the juice can be heated only to, say, 170°, making it possible to utilize an amount of vapor from the third body of, say, 6%. The temperature of the vapor from the first body is

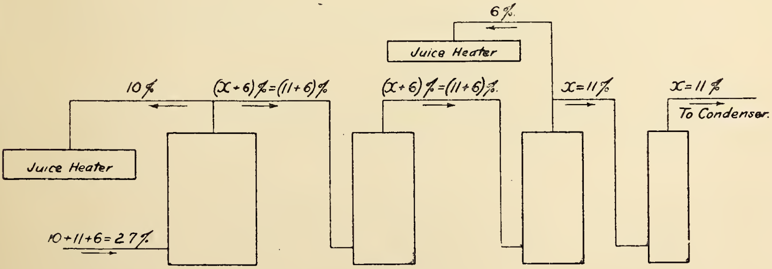

FIG. 2.

sufficiently high to do the remaining 10% of heating. Here again let

X = the per cent of vapors formed in the last body.
Then the total evaporation in the third body will be X+6%,
That in the second body X+6%,
While that in the first body must be (X+6+10)%.
The total evaporation will be (4X+6+6+6+10)%=72%
X= 11%

The evaporation in the three bodies will therefore be as follows:

First body	27%
Second body	17%
Third body	17%
Fourth body	11%
Steam required for first body	27%
The total for evaporation and heating will be	27%

It will be noted that this gives a saving still greater than that for the arrangement of Figure 1.

The two examples given above will serve to show the method of determining the total steam consumption for heating and evaporating where vacuum juice heaters are used. Following are a number of other possible arrangements of vacuum heaters for which the steam consumption for heating and evaporating has been calculated by the general method illustrated above.

CASE 3.

Quadruple Effect.

Juice heated from 82° to 192° requiring 14% vapor from second body, the remaining 2% of heating to be done with direct (boiler or exhaust) steam:

Steam supplied to first body	25%
Total steam for heating and evaporating	25+2= 27%

CASE 4.

Quadruple Effect.

Juice heated from 82° to 172° with vapor from third body, requiring 10% vapor, the remaining 6% of heating to be done with direct steam:

Steam supplied to first body........................... 20.5%
Total for heating and evaporating.............20.5+6=26.5%

CASE 5.

Juice heated from 82° to 132° with vapor from last body, requiring 6% vapor, the remaining 10% heating to be done with direct steam. Note that vapors with a temperature of 140° can only raise the temperature to about 132°:

The steam required for first body........................ 18%
Total steam for heating and evaporating........18+10= 28%

CASE 6.

Triple Effect.

Temperature of boiling 208°, 182°, 140° in first, second and third bodies respectively.

Due to the fact that the temperature in the first body is 208°, it will be impossible to raise the temperature to 212° with vapor from that body. Hence 14% of the heating will be done with vapor from the first body and the remaining 2% with direct steam.

Steam required for first body........................ 33⅓%
Total steam for heating and evaporating.....33⅓+2= 35⅓%
Note that with a plain triple, the total for heating and evaporating would be........................... 40%

CASE 7.

Triple Effect.

Use 6% vapor from last body for one heater and 10% from first body for another heater.

Steam required for first body........................ 30⅔%
Total steam for heating and evaporating............... 30⅔%

CASE 8.

Double Effect.

Temperature of boiling 194° and 140° in the first and second bodies respectively.

Use 12% vapor from first body for heating, the remaining 4% heating to be done with direct steam.

Steam required for first body........................... 42%
Total steam for heating and evaporating..........42+4=46%

Note that for a plain double effect, the steam for heating and evaporating is 52%.

CASE 9.

Double Effect.

Use 6% vapor from second body in one heater and 10% vapor from first body.

Steam required for first body	41%
Total steam for heating and evaporating	41%

Evidently there are many other possible combinations that could be arranged, depending upon the conditions. By comparing the steam requirements for the different cases given above, it will be readily seen that a given amount of vapor used from later bodies will result in a greater saving of steam than when taken from earlier bodies. For example, the total steam consumption for heating and evaporating with 6% vapor taken from the different bodies of a quadruple effect under the general conditions used heretofore will give the following total steam consumed for heating and evaporating:

Plain quad	34%
6% from first body	32½%
6% from second body	31%
6% from third body	29⅔%
6% from fourth body	28%

Referring to CASE 1 and CASE 2, it will be noted that the use of vapor heaters not only reduces the steam consumption but the amount of steam entering the condenser as well. In other words, an installation having vacuum juice heaters would require a smaller condenser than where a plain effect is used. Another important point in connection with vacuum juice heaters is that, although the greatest theoretical saving is effected by using vapor from later bodies, the cost of equipment will be greater where vapor is taken from the later bodies. This is due to the fact that in the last body the temperature fall is relatively low. For example, consider the first and last body of a quadruple effect. Assume that the juice can be raised to a temperature within 4° of the heating vapor. In the first body the juice can be raised from 82° to 210°, average 146°, and the temperature fall will be 216—146=170°. With vapor from the fourth body the juice can be raised from 82° to 146°, average 114°, and the temperature fall will be 140—114=26°. In other words, the temperature fall in the last body heater is less than one-sixth that in the first body heater, consequently the last body heater would require more than six times the heating surface in the first body heater to do the same amount of heating.

In view of the greater specific volume of the vapor in later bodies, it is evident that the vapor pipes to heaters for the later bodies must be much larger than those for earlier bodies. The later bodies also present other difficulties, such as a greater amount of leakage which is likely to result in lower coefficients of heat transmission due to the air in the steam, also to lower steam densities.

The present practice is to construct a multiple effect with the same amount of heating surface per body. However, when vacuum heaters are used this is not the case, since additional heating surface has to be used in the bodies supplying vapor for heating. For example, in Case 1 the evaporation in bodies 1, 2, 3 and 4 are 30%, 14%, 14% and 14% respectively and the heating surfaces in them should be in the same proportion. This is shown graphically in Figure 1 and Figure 2, in which the relative evaporation and area of heating surface is indicated by the area of the diagrams representing the different bodies. In Case 1 the last three bodies would have equal heating surface, but the first one would have a larger heating surface. In Case 2, the evaporation in the bodies is 27%, 17%, 17% and 11%, respectively, hence their heating surfaces should be in the same proportions. Naturally the total cost of evaporator heating surface with such an arrangement may in some cases be greater than in a plain quad having equal heating surface per body. In this connection, it may be noted that vapor may be taken from the last body for heating without affecting the equality of heating surface.

INTRODUCTION.

The experiments on vacuum juice heaters were made upon three different apparatus, namely: (1) an especially designed small juice heater connected with a single effect evaporator in the engineering laboratory; (2) vacuum juice heaters connected to each body of the double effect at the Audubon Park sugar factory, and (3) the vapor juice heaters in connection with the quadruple effect at Central Florida in Cuba.

The first of these three apparatus was used strictly for research experiments, whereas the other two may be considered as operating under regular factory conditions.

EXPERIMENTS ON THE LABORATORY JUICE HEATER.

As stated, this heater was designed especially for research work and so that conditions of operation could be more perfectly controlled than would be possible in regular factory heaters. This apparatus is shown in Figure 3. The body of the heater (H) was made up of six-inch pipe and fittings; the heating surface consists of one tube (cc), through which the juice passes, with a stuffing box at each end to provide a tight joint and still allow for expansion. The apparatus was arranged so that tubes of varying sizes or varying materials could be used conveniently, though, so far, tubes of ¾″ outside diameter only have been used. The length of tube subject to contact with hot steam is 42 inches. Steam is admitted to the heater midway between the two ends either under pressure or vacuum. Steam under pressure is admitted through valve E and steam under vacuum through valve D. Steam under vacuum is obtained from the small vacuum evaporator situated near the heater. The manner of connecting the evaporator to the heater may be understood by referring to Figure 4, the pipe marked D supplying vapor to the heater. By properly operating the evaporator, vapor under any desired vacuum can be supplied. Incondensable gases entering the evaporator by leakage would, of course, result in a certain amount of air reaching the heater, which is vented through ¼″ pipes (ff) at each end of the heater to a vacuum pump. Condensation is drawn off from the bottom of the heater at the two points gg and is drained into the receiver (F), which is 6″ in diameter and about 42″ long. This receiver is provided with a gauge-glass (G) by means of which volumetric measurements of the amount of condensation can be made. In order to insure that the condensation will drain into this receiver, an equalizing pipe

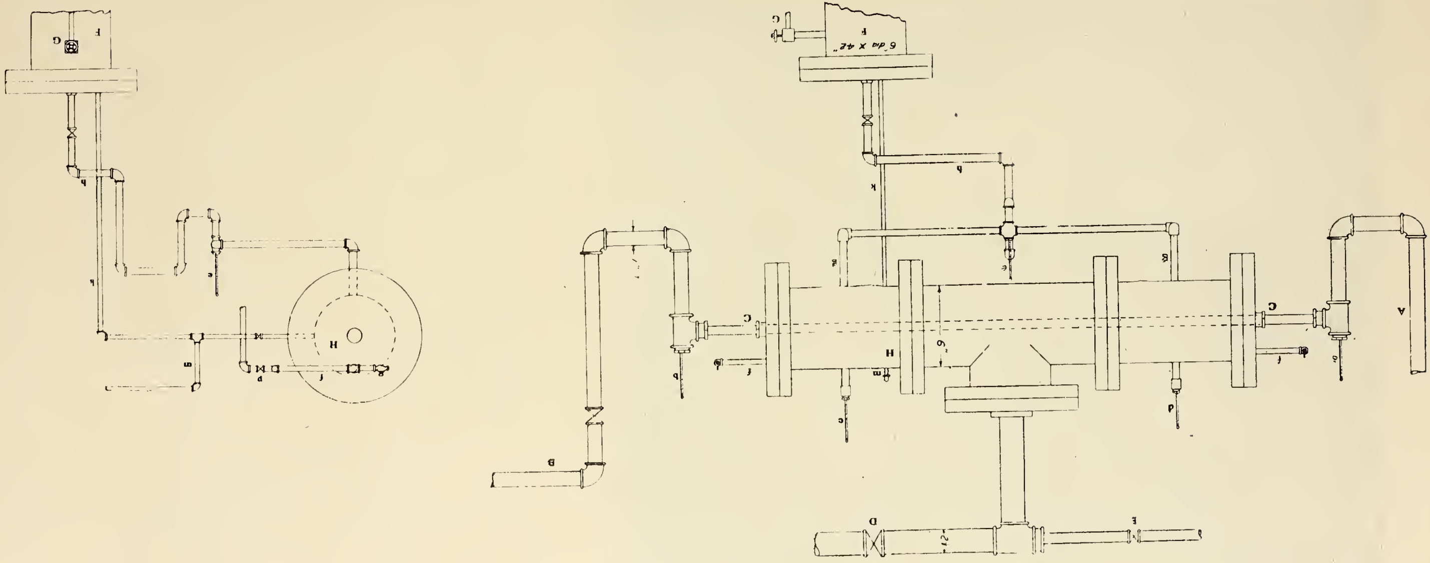

FIG. 3.

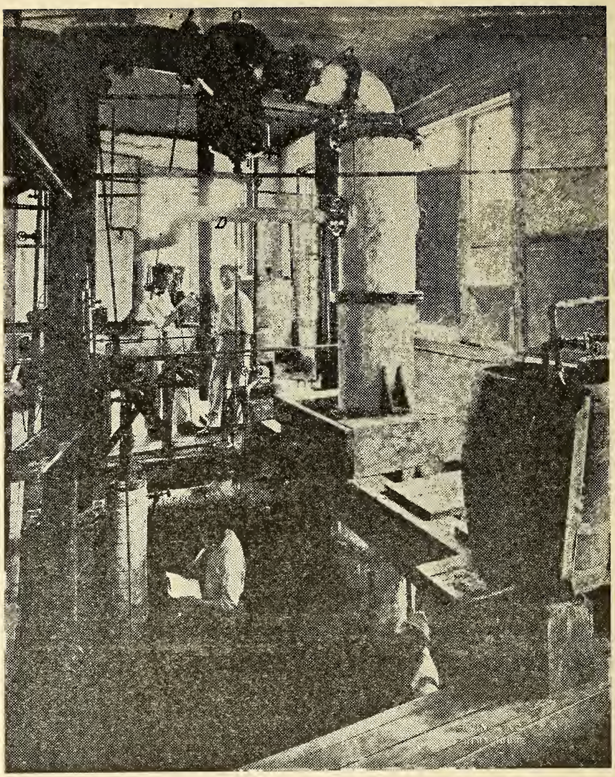

FIG. 4.

(k) ¼″ in diameter, connecting the heater and the receiver, is provided. Juice enters the apparatus at (A) and leaves at (B). The temperatures of the juice entering and leaving were determined by thermometers (a) and (b). Thermometer (e) was used for determining the temperature of the condensate leaving the heater. The temperature of the steam in the heater was determined by two thermometers (b) and (c). The vacuum in the heater was measured by means of a mercury column gauge. When steam above atmospheric pressure was used, a spring gauge was used for determining its pressure. The body of the heater and the receiver were covered with cement and asbestos 1½″ thick. The connecting pipes were also covered.

PROCEDURE.

The prime object in most of the experiments made on this heater was to determine the coefficient of heat transmission. The amount of condensation drained into the heater was used as a basis for all calculations regarding heat transmission. Of course, the condensation reaching the receiver was not only that due to heat actually transmitted but to radiation as well. In order to determine the amount of condensation resulting from heat transmission alone, it was necessary in each test to first determine the condensation due to radiation alone and then subtract it from the total condensation. This required special tests to determine the condensation due to radiation for each steam temperature. In some test series, constant steam temperatures were used, in which case one radiation test only was required for the series.

In starting up a test, the following procedure is typical: water or juice was first started slowly through the heater and steam turned on to warm it up. The vent valve (p) was then opened and regulated so as to get the desired mixture of air and steam. In doing this, thermometers (b) and (c) were used as a guide. For a given steam presure or vacuum, there is a certain temperature for air-free steam. Since it is practically impossible to secure air-free steam, it was seldom that the temperatures shown by these thermometers were as high as the steam table temperature corresponding to the pressure. By increasing the amount of vent opening, temperatures recorded by these thermometers could be made to approach those for saturated steam at the pressure, or by reducing these openings the temperatures shown by these thermometers could be correspondingly reduced. Curves showing the relation between steam temperature and absolute pressure were plotted and kept in use for reference while the heater was being operated, which made it possible to regulate the vents in a convenient manner. After the conditions required for the test were obtained in the manner described, the height of condensation in the receiver was noted and the test started. The difference between the height of the water in the receiver at the end and at the beginning of the test gave the volume condensed, from which the heat transmission calculations were made. In most of the tests the following observations were made: Temperatures of steam, juice inlet, juice outlet, air in room, condensation, steam pressure or vacuum and barometer. As will be noted, the duration of the tests varied from 16 minutes to 48 minutes, the average being about 30 minutes. The duration of the test was in practically all cases determined by the length of time required to fill the receiver with condensate.

METHOD OF CALCULATING THE RESULTS.

The coefficient of heat transmission—that is, B. T. U.—transmitted per square foot of heating surface per hour per degree difference in temperature (Fahr.) was determined by the formula

$$C = \frac{(W-w)\ L}{T \times S\ (t-t_1)} \text{ where}$$

C = coefficient of heat transmission;
W = weight of condensation during test;
w = condensation due to radiation;
L = latent heat corresponding to the steam pressure maintained in the heater during the test.
T = duration of test in hours;
S = heating surface in square feet;
t = temperature of steam;
t_1 = average temperature of juice.

There are two methods for determining the average temperature of the juice. An approximate method gives:

$$t_1 = \frac{t_2 + t_3}{2} \text{ where}$$

t_2 equal temperature of juice entering heater
t_3 equal temperature of juice leaving heater

A more accurate value is given by the formula:

$$t - t_1 = \frac{t_3 - t_2}{\log._e \dfrac{t - t_2}{t - t_3}}$$

Since heat transmission is affected by the velocity of the juice through the heating tubes, it was necessary to exercise some control over the juice velocity. In all tests, a carefully calibrated juice meter was used, from which the velocities were calculated. The velocity

$$V = \frac{N}{A} \text{ where}$$

V = velocity, F. P. M.

N = flow, cubic feet per minute (from meter readings).

A = cross-sectional area of tube, square feet.

According to Dalton's law, the pressure of the mixture of air or other gases and a vapor is the sum of the pressure each would have if it occupied the same space alone. In other words, the pressure or vacuum gauge attached to the compartment containing steam shows the sum of the pressures due to steam and air. The temperature of the steam is that corresponding to its pressure. Hence, if the temperature of the mixture is known, it is easy to find the partial pressure of the steam by referring to a steam table. By determining the pressure due to the steam in this manner and subtracting it from the sum of pressures, as shown by the gauge, we obtain the pressure due to the air.

Let P_t equal the total pressure of air and steam (gauge pressure).

Let P_s equal partial steam pressure as explained before.

Then $\frac{P_s}{P_t}$ is a ratio which is proportional to the richness of the (steam-air) mixture. For air-free steam, its value would evidently be —1—. With air present, it will always be less than —1— and the greater the amount of air the smaller its value.

Since the determination of the amount of air in the mixture by this method depends upon the correct measurement of the temperature, especial care was used not only by submerging them properly but by using thermometers of a very high grade and graduated to 1/10 degree.

SCOPE OF THE EXPERIMENTS.

Originally it was intended to carry out four series of tests to determine as follows:

1. The effect of air in steam on heat transmission with which is closely associated the method of venting.
2. The effect of juice velocity on heat transmission.
3. The effect of the material of which the heating tubes are made upon heat transmission.
4. The effect of scale on heat transmission.

Up to the present time, only the first three of these have been done.

RESULTS OF THE EXPERIMENTS.

Table 1 gives data which is representative of those derived from the tests. This table is divided into three parts: (A), (B) and (C), (A) showing the results of the "air-in-steam" series, (B) the "juice-velocity" series and (C) the "tube-material" series.

These tables do not by any means include all of the tests that were made. Many preliminary experiments were made previous to those given in the table in order to learn the proper control of conditions necessary in order that the effect of any one variable could be ascertained.

AIR IN STEAM.

When the heater was first started considerable difficulty was encountered in getting rid of and in controlling the air in the steam compartment of the heater. The venting was controlled by means of a ¼″ valve in the vent pipe. The measurement of the amount of air in the air-steam mixture was ascertain accurately through the use of very delicate and accurate thermometers. An approximate guide as to the thoroughness with which the venting was accomplished was the number of turns the vent valve was open. Sometimes the relation between the "number of turns open" to "temperature drop"was very erratic. It was found, however, that this could be much improved by opening the vents wide for a short time after steam was turned on at the beginning of a test.

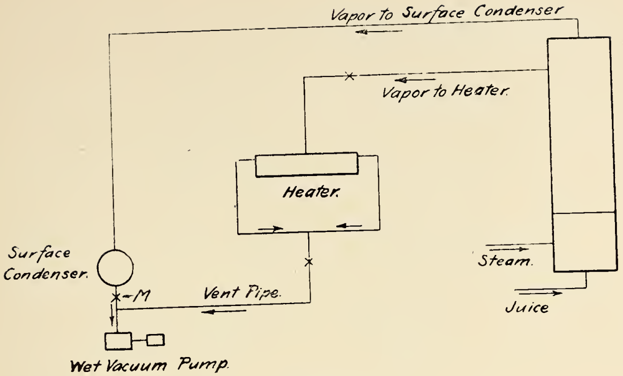

FIG. 5.

This voided the heater of air, after which the valves were set so as to give the temperature which indicated the desired air richness, which temperature could generally be maintained constant.

The method of drawing off the air by means of the wet vacuum pump, which serves the surface condenser, is shown in the diagram of Figure 5. This diagram also shows the method of supplying vapor to the heater from the vacuum evaporator heretofore mentioned. It will be noted that some of the vapor from the evaporator passes to the surface condenser, where it is condensed and drawn off by the wet vacuum pump. A small proportion of the evaporator vapor, however, goes to the experimental heater. When the tests of the heater were first started, there was much trouble in venting the steam compartment, due to the fact that there was no pressure fall from the heater to the vacuum pump. In order to overcome this, the ¼″ vent pipe was connected to the suction of the pump below the valve "M." By throttling valve "M," it was possible to get a greater vacuum below the valve than that in the heater itself. By this means, the air and other gases could be drawn from the heater. It will be noted that the difference in vacuum in the tests varied from about 2″ up to 4″ (see columns 6 and 7, Table 1).

TABLE 1.

Test No.	Duration of Test, Min.	Temp. of Steam	Temp. Juice Inlet	Temp. Juice Outlet	Vacuum in Heater, Ins. Mer.	Vacuum at Pump Suction	Barometer	Condensate, lbs.	Juice, lbs.	Juice Vel. F. P. M.	$\frac{Ps}{Pt}$	Coef. Heat Trans.	Remarks
"A"—"AIR IN STEAM SERIES."													
1	8	148.5	88.4	88.8	9.81	14.5	30.18	Trace	392	370	.359	....	Vent valves open 1/10 turn.
2	48	185.4	88.9	92.2	10.34	14.5	30.18	5.97	1853	291	.869	88.3	Vent valves open ¼ turn.
3	16	191.1	89.2	102.3	10.62	14.0	30.18	8.74	624	295	.906	502.6	Vent valves open ½ turn.
4	32	188.3	89.0	96.8	10.51	13.5	30.18	10.37	1250	295	.934	300.5	Vent valves open ⅜ turn.
5	16	190.8	89.0	100.7	10.43	12.5	30.18	7.7	618	292	.980	448.0	Vent valves open ⅝ turn.
6	16	191.2	89.3	101.5	10.19	12.5	30.18	7.53	596	281	.978	449.0	Vent valves open ¾ turn.
7	16	190.3	89.5	101.0	10.58	13.0	30.18	7.53	617	292	.975	430.0	Vent valves open ⅞ turn.
"B"—"JUICE VELOCITY SERIES."													
8	16	193.0	88.9	111.6	9.99	13.5	30.23	7.19	280	131.3	1.0	411.9	Vent valves open 1 turn.
9	16	193.3	88.5	108.2	9.77	13.0	30.24	7.97	382	179.3	1.0	446.9	Vent valves open 1 turn.
10	16	192.4	88.5	104.2	10.12	13.0	30.25	7.97	514	242.0	1.0	450.3	Vent valves open 1 turn.
11	16	190.7	88.5	103.8	9.73	13.5	30.27	9.31	616	289.3	.94	548.2	Vent valves open 1 turn.
12	16	189.3	88.6	101.7	10.07	13.5	30.27	9.31	708	332.0	.93	540.5	Vent valves open 1 turn.
13	16	192.8	88.6	100.0	9.90	13.5	30.25	10.47	862	405.0	.992	572.7	Vent valves open 1 turn.
14	16	193.0	88.7	99.2	9.73	13.5	30.23	10.72	1070	502.0	.995	587.8	Vent valves open 1 turn.
"C"—"COPPER TUBE SERIES."													
15	16	210.7	89.0	110.9	0.0	4.0	29.87	17.01	....	....	.975	814	Vent valves open ¼ turn.
16	24	197.1	89.0	100.3	6.65	9.5	29.87	15.12	....	....	.951	530	Vent valves open ½ turn.
17	16	182.6	89.0	98.7	12.94	16.0	29.87	7.43	....	....	.956	450	Vent valves open ¾ turn.
18	24	166.4	89.0	96.2	18.10	21.0	29.91	9.72	....	....	.944	503.5	Vent valves nearly wide open.
STEEL TUBE.													
19	16	210.8	89.5	104.3	0.0	4.0	29.91	10.26	....	....	.975	474	Vent valves open ¼ turn.
20	24	197.8	89.1	95.3	6.62	9.5	29.91	8.24	....	....	.964	255	Vent valves open ½ turn.
21	20	176.8	89.0	93.5	13.08	16.0	29.91	5.00	....	....	.864	244.5	Vent valves wide open.
22	16	164.0	90.0	93.6	17.38	21.0	29.91	2.03	....	....	.847	148.0	Vent valves wide open.

Reference to "A" of Table 1 shows the marked effect of the presence of air in steam in reducing the coefficient of heat transmission. It will be noted that the vacuum in most of these tests was in the neighborhood of 10″, the corresponding temperature of steam being approximately 190°, which is about what would be found in the second body of a quadruple effect. It will be remembered that $\frac{P_s}{P_t}$ is a measure of the air richness of the mixture, the smaller the ratio the greater the amount of air. In Test No. 1, with a value of $\frac{P_s}{P_t} = .359$, there was practically no transmission of heat. In this case, the vent valves were opened only 1/10 of a turn. The results of the tests are shown graphically in the straight line curve of Figure 6. The test points are remarkably close to the average curve. As stated heretofore, this series was made after a considerable amount of preliminary work and the results should be dependable. This shows the necessity of properly venting vacuum juice heaters. In this regard, it may be stated that where a plunger or piston wet vacuum pump is used for removing the condensate from a vacuum heater, most of the incondensable gases can be drawn off by the same pump. In the case of a centrifugal pump, however, separate vents, preferably connected to the top of the steam compartment, are neces-

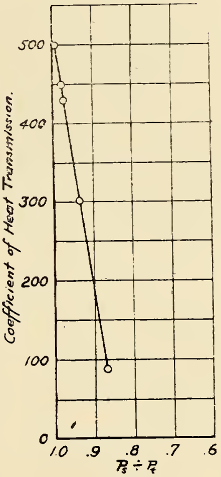

Fig. 6.

sary, since centrifugal pumps will not remove air. The need of venting is especially important where high vacuums are used. In the case of heaters operating with pressures above that of the atmosphere, the accumulation of air or other incondensable gases is small and the effect still less because of the greater steam densities.

It will be well to call attention at this point to the fact that the heater upon which these tests were made is not well designed for the removal of incondensable gases, for the reason that the steam compartment is too large compared with the area of the heating surface; that is, the diameter of the steam compartment is 6″ and that of the heating tube ¾″, the result being that there is practically no velocity of steam flow. If the steam compartment had been, say, 2″ in diameter with the same diameter (¾″) of heating tube, and if the steam had been admitted at one end instead of at the middle, with the vent at the opposite end, there would have been not only a more rapid steam flow but better separation of air from steam. In other words, the longer and narrower the steam path the greater the steam velocity, the better the separation of air from steam and the higher the coefficient of heat transmission. In future experiments a heater of this type will be used.

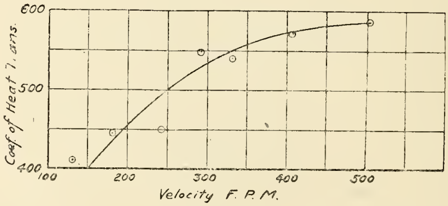

Fig. 7.

The results of the juice velocity tests are shown in "B" of Table 1. The results are also shown in the curve of Figure 7. The juice velocity was varied from a minimum of 131 F. P. M. to a maximum of 502 F. P. M., with corresponding variations in the coefficient of heat transmission from 412 to 588.

The fact of the matter is that much research has already been done along this line by other experimenters. However, the experiments were easily carried on with this apparatus, which accounts for the fact that a somewhat overdone field was entered.

The importance of high velocities in vapor juice heaters is greater than for heaters having pressure, especially as the tendency is towards low coefficients of heat transmission, due to various reasons that will be touched upon in this bulletin. High juice velocities may be obtained in design by using long tubes of small diameter and multiple passes. High juice velocities not only increase the rate of heat transmission but serve, by the scouring action thus produced, to prevent fouling of tubes. Frequently juice heaters are required to operate at capacities lower than that for which they were designed, the result being that the juice velocities are low and the heaters work badly. Frequently this difficulty can be overcome by plugging some of the tubes in each pass.

"C" of Table 1 gives the results of the tests made to determine the comparative coefficients of heat transmission for copper, and steel, tubes. The copper tube used was the same as that used for Tests 1 to 14, inclusive. The tube used in Tests 19 to 22, inclusive, was a Shelby seamless cold-drawn steel tube of the same dimensions as the copper tube. Incidentally, these tests (C) furnish some data regarding the coefficients of heat transmission with varying pressure (vacuum) of the steam. Four tests were made on each tube, the vacuum carried being approximately 0.0″, 6″, 13″ and 18″, respectively, for each material. The juice velocity was the same in all tests. The coefficients are also platted in the two curves of Figure 8. The average coefficients for the copper and steel tubes were 576 and 280, respectively. It will be noted,

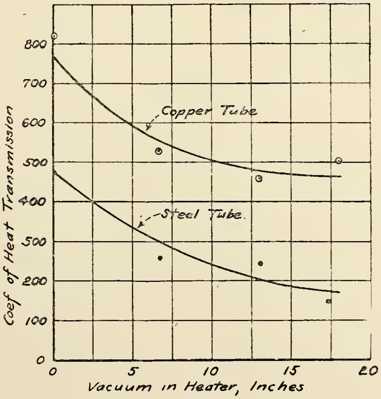

FIG. 8.

however, that the conditions were less favorable for the steel tube, the average value of $\frac{P_s}{P_t}$ being .908, whereas it was .956 for the copper tube. Correcting the coefficient 280 for $\frac{P_s}{P_t} = .95$ (see Figure 4), we have 430. This value is about 25% less than that for the copper tube. This comparison being made with perfectly clean tubes, does not, perhaps, give a fair comparison for tubes slightly coated with scale as found in practice. It is probable that the difference of 25% would disappear altogether with fouled tubes, since in that case the principal resistance to the flow of heat is offered by the scale, the conducting power of the metal being sufficient to transmit even more heat than can reach it through the scale. Referring again to Figure 6, it will be noted that the curves incline downward to the right; that is, the greater the vacuum under which the steam exists the lower the coefficient of heat transmission. This may be due in part to the decreased density of steam, also in part to the greater amount of air at the higher vacuum. It will be noted that the value $\frac{P_s}{P_t}$ greatly decreases with increased vacuum in tests on both the copper tube and the steel tube.

EXPERIMENTS ON THE VACUUM JUICE HEATERS AT THE AUDUBON PARK SUGAR EXPERIMENT STATION.

Figure 9 shows the apparatus used in these experiments. The double effect is one that is used regularly for evaporating juice during the grinding season at the sugar factory operated by the Sugar Experiment Station of the Louisiana State University. This factory is operated by students of the sugar course of the Louisiana State University. The vacuum heaters (A) and (B) were arranged and constructed especially for these experiments.

Figure 10 shows a sectional view of one of these heaters. Steam enters at —e—, passes to the bottom of the steam chamber and

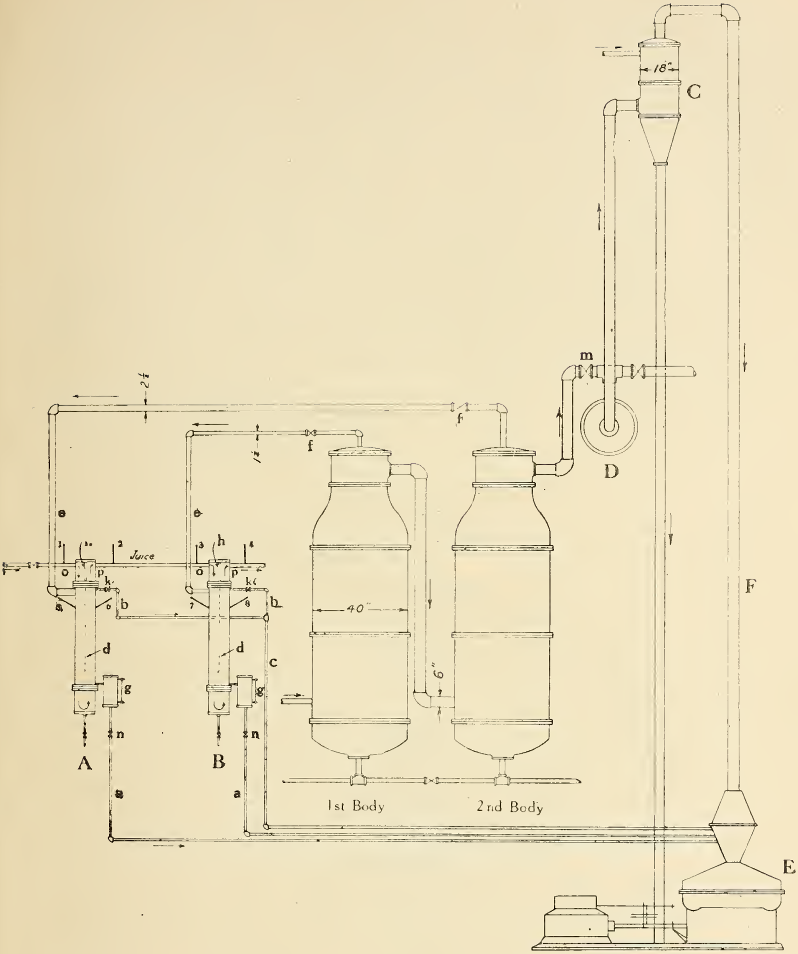

Fig. 9.

around the baffle —d—, the incondensable gases being drawn off at —b— the end of the steam path. Juice enters at —o—, passes through one set of tubes and up through another set and out at —p—. Thus, it will be noted, both steam and juice have double passes through each heater. The baffle —h— effects the double passage for the juice. Each heater has eight tubes, 1¼" outside diameter by 42½" long, making a total of 9.26 sq. ft. of heating surface in each heater.

Referring to Figure 9, it will be noted that both heaters are connected by pipes —e— to the tops of the first and second bodies respectively of the double effect, consequently the temperature and vacuum in heater (A) should be practically the same as that in the vapor space of the first body, while that in heater (B) should be the same as in the vapor space of the second body. The cold juice enters the heater with coolest vapor—namely, (A)—first, after which it passes to heater (B) with hotter vapor, where its temperature is still further increased. The vent pipes —b—, each with controlling valve —k—, are connected to pipe —c—, which, in turn, is connected to the vacuum pump E. The condensation is drained through valves —n— and pipes —a— to the vacuum pump E. In order to prevent the blowing of steam through these pipes receivers —g— with gauge glasses were attached. By regulating the valves —n— so as to keep water showing at the proper height in these gauge glasses, no steam could blow through. The vapor leaving the second body of the

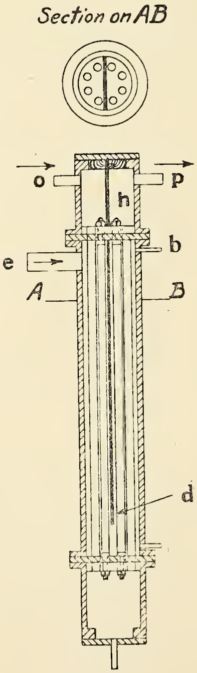

FIG. 10.

double effect passes first through valve —m—, then through the cylindrical receiver —D— and finally to the barometric condenser C. The air is drawn by the vacuum pump from the top of the condenser through pipe F. The positions of thermometers used are indicated in the figure. Thermometers 1, 2, 3 and 4 were used for measuring juice temperatures and thermometers 5, 6, 7 and 8 for vapor temperatures. The thermometers for juice were immersed in wells, whereas those for vapor were projected through rubber bushings directly into the vapor. In experiments made at the beginning, the vacuum in the vapor compartments of the heaters was determined by means of vacuum gauges attached to the vapor spaces of the bodies of the double effect. In most of the tests, however, mercury column vacuum gauges were attached to the heaters themselves.

PROCEDURE.

While the objects sought in the tests on this apparatus were similar to those for the tests on the laboratory heater, the fact that the vacuum heaters were connected up to the bodies of the evaporator used in the regular operation of the sugar factory, made it impossible to exercise the accurate control that was possible with the laboratory heaters. The principal objects of the tests was to observe the factors affecting the operation of such heaters and to determine as far as possible what was necessary in order to get the most satisfactory results as regards capacity. Briefly, the procedure in all tests was first to start the double effect; after it was operating normally to start juice through the heaters, then open valves —f—, which admitted vapors to the heaters. In order to void the steam compartment of air or other incondensable gases, vent valves —k— were opened wide at the beginning and then gradually closed to the required point. In regulating these valves, the temperature recorded by thermometer 6 for heater "A" and thermometer 8 for heater "B" was used as a guide. In the case of heater "A", if the temperature shown by thermometer 6 was much lower than that shown by thermometer 5, the vent valve was opened an additional amount, etc. When thermometer 6 showed the same temperature as thermometer 5, it was assumed that the venting was satisfactory; the same was true of thermometers 7 and 8 for the other heater. There was considerable difficulty in getting a proper venting of heater "A." Evidently the vacuum in the vapor compartment of this heater was practically equal to that in the vapor space of the second body, and, for that matter, at the suction of the vacuum pump. In other words, there was no pressure drop that would cause the flow of air through the vent pipe —c— to the vacuum pump. This was partially overcome in some of the tests by throttling the 6″ valve —m— in the vapor pipe between the second body and the condenser. By this means it was possible to get a greater vacuum at the suction of the vacuum pump than in the vapor space of the second body, or, which is the same thing, than in the vapor compartment of heater "A." It should be stated, however, that the venting was always erratic and unsatisfactory in the case of heater "A." There was difficulty in securing steady operation of the effect itself and this, in turn, made it difficult to maintain the necessary drop of pressure from the heater to the vacuum pumps.

The accumulation of air in both heaters, and especially in "A," was very noticeable and the heater very sensitive in this respect. Every precaution was taken to provide against leakage of air into the effects, heaters, and connecting pipes. However, there was probably a small amount of leakage. If the vent valves were not open sufficiently, that part of the steam compartment at the end of the steam path would begin to cool in some cases very rapidly and this could be easily detected by placing the hand on the shell of the heater at this point.

Heater "B," however, gave little trouble in this respect, due mainly to the fact that there was considerable pressure drop from the heater to the vacuum pump. Considerable difficulty was also encountered in removing the condensation from heater "A" for the same lack of pressure fall, whereas with heater "B" there was no difficulty whatsoever.

As in the tests on the laboratory heater, all observations necessary for determining the coefficient of heat transmission, the air in steam, etc., were made. The methods used were practically the same as in the laboratory tests, except that juice instead of condensation was weighed, and this was used as a basis for the heat transmission calculations. During the tests observations were made every five minutes.

METHOD OF CALCULATING RESULTS.

The coefficient of heat transmission was calculated according to the formula:

$$C = \frac{J \times (t_5 - t_4) \times k}{T \times S\ (t - t_1)} \quad \text{where}$$

C = coefficient of heat transmission;
J = weight of juice fed during test, in pounds;
t_4 = temperature of juice entering;
t_5 = temperature of juice leaving;
k = specific heat of juice;
T = duration of test, hours;
S = heating surface, square feet;
t = temperature of steam;
t_1 = average temperature of juice.

The value of $(t - t_1)$ was calculated in the manner described on page 14. It should be stated here also that the actual and not the temperature corresponding to the vacuum was used for the value of t.

The juice velocity was calculated acocrding to the formula:

$$V = \frac{J}{D \times A} \quad \text{where}$$

D = density of juice, pounds per cubic foot;
A = cross-sectional area of the tubes in one pass.

The value of $\frac{P_s}{P_t}$ was calculated as described for the laboratory heater tests, the steam temperature used being the average from the thermometers 5 and 6 for heater "A" and thermometers 7 and 8 for heater "B."

SCOPE OF THE EXPERIMENTS.

Attention has already been called to the fact that the conditions of operation in these tests were not under perfect control, due to the fact that the apparatus was used in the regular operation of the factory. The objects of the tests, therefore, were as much for observing the general behavior of the apparatus as for securing heat transmission data. However, the experiments included observations that were intended to make it possible to study the effect of air in steam, juice velocity, amount of vacuum and the use of juice as compared with water upon the coefficient of heat transmission.

TABLE 2.

1	2	3	4	5	6	7	8	9	10	11	12	13	14
Test	Duration of Test—Minutes	Temperature Juice Entering "A" Heater	Temperature Juice Leaving "A" Heater	Temperature Juice Enter-in "B" Heater	Temperature Juice Leaving "B" Heater	Vacuum, "A" Heater	Vacuum, "B" Heater	Barometer	Temperature Vapor, "A" Heater, Near Vapor Entrance	Temperature Vapor, "A" Heater, Near Vent.	Temperature Vapor, "B" Heater, Near Vapor Entrance	Temperature Vapor, "B" Heater, Near Vent	Temperature Rise of Juice in "A" Heater
1.........	20	68	70.6	70.3	91.3	23.2	16.3	29.2	124.4	91.4	161.1	148.3	2.6
2.........	20	70.3	77.7	77.5	113.7	24.9	14.2	29.92	130.2	105.3	176.9	158.0	7.4
3.........	20	65.4	71.3	71.3	109.0	23.5	11.9	30.10	113.0	120.2	175.5	176.7	5.9
4.........	20	71.6	83.8	83.8	116.4	25.2	17.6	30.10	127.0	110.1	170.4	160.2	12.2
5.........	20	66.6	86.4	86.4	119.8	22.96	14.8	30.14	146.2	114.8	178.7	162.1	19.8
6.........	25	66.1	92.0	92.0	124.2	22.2	15.1	30.14	142.0	134.0	175.5	159.5	25.9
7.........	25	68.3	87.0	87.0	113.3	24.0	15.7	30.14	133.0	131.6	169.3	161.3	18.7
8.........	10	69.3	84.0	83.0	109.0	25.6	13.2	30.14	130.6	122.0	180.8	172.4	14.7
9.........	20	67.4	98.8	98.7	132.3	21.1	13.51	30.08	156.6	154.4	177.9	170.2	31.4
10.........	15	76.8	93.7	93.8	135.0	20.7	9.41	30.08	131.2	128.3	189.2	184.1	16.9
11.........	10	....	....	72.0	122.0	....	8.85	30.08	....	....	199.9	199.0	....
12.........	10	....	....	72.3	116.5	....	10.2	30.08	....	....	190.6	187.3	....
13.........	15	....	....	80.0	116.5	....	13.6	30.08	....	....	181.9	178.2	....
14.........	15	....	....	86.4	115.8	....	17.3	30.08	....	....	171.9	167.4	....
15.........	15	....	....	69.0	98.0	....	20.8	30.08	....	....	155.5	151.3	....
16.........	128	67.2	102.9	103.1	137.9	19.76	12.36	30.02	155.6	147.1	183.5	176.5	35.7
17.........	67	73.8	103.2	102.3	136.8	15.42	6.68	30.02	176.6	176.5	199.0	194.9	29.4
18.........	52	76.3	95.9	95.1	117.9	20.45	11.43	30.02	158.9	157.6	187.7	183.0	19.6
19.........	42	60.0	70.0	69.0	94.8	22.43	....	30.11	145.0	123.6	182.8	175.1	10.0
20.........	33	64.3	80.6	81.2	105.8	21.96	....	30.11	151.8	144.1	190.0	181.7	16.3

TABLE 2—Continued.

	15	16	17	18	19	20	21	22	23	24	25	26	27
Test	Temperature Rise of Juice in "B" Heater	Average Observed Temperature of Vapor "A" Heater	Average Observed Temperature of Vapor "B" Heater	Temperature Corresponding to Vacuum "A" Heater	Temperature Corresponding to Vacuum "B" Heater	Juice Velocity F. P. M.	Coefficient Heat Transmission Using Temperature Due to Vacuum "A" Heater	Coefficient Heat Transmission Using Temperature Due to Vacuum "B" Heater.	Coefficient Using Actual Temperature of Vapor "A" Heater	Coefficient Using Actual Temperature of Vapor "B" Heater	$\frac{P_s}{P_t}$ "A" Heater	$\frac{P_s}{P_t}$ "B" Heater	Juice Fed During Test, lbs.
1..........	21.0	107.9	157.2	145.3	174	51	....	127.8	....	157	.36	.66	1752
2..........	36.2	117.7	166.1	134.0	181.2	52	71.8	250.0	99.0	369	.64	.71	1799
3..........	37.7	116.6	176.1	144.6	188.0	50	43.3	219.0	68.6	250	.46	.79	1733
4..........	32.6	118.6	165.3	133.0	171.0	41	100.0	212.0	137.6	233	.68	.88	1397
5..........	33.4	130.5	175.4	148.0	180.1	46	142.7	228.0	186.7	242	.65	.90	1580
6..........	33.2	138.0	167.5	152.0	176.2	38	152.0	202.0	189.0	233	.70	.77	1616
7..........	26.3	132.3	165.3	141.7	177.4	64	208.7	484.0	246.0	291	.78	.76	2740
8..........	26.6	126.3	176.6	130.0	184.6	86	267.0	292.0	286.0	321	.90	.88	1485
9..........	33.6	155.5	174.0	157.1	183.7	50	240.3	281.3	251.0	328	.96	.81	1722
10..........	41.2	129.7	186.6	158.6	193.9	52	98.1	155.8	99.0	174	.48	.85	1311
11..........	50.0	....	199.5	....	195.2	47	....	268.0	....	258	....	1.00	795
12..........	44.2	....	188.6	....	192.0	57	....	237.3	....	246	....	.93	962
13..........	36.5	....	180.0	....	183.4	62	....	271.5	....	284	....	.93	1570
14..........	29.4	....	169.6	....	172.0	51	....	235.0	....	244	....	.95	1292
15..........	29.0	....	153.4	....	157.6	66	....	221.3	....	234	....	.89	1280
16..........	34.8	151.4	180.0	162.6	186.6	13	76.9	82.2	....	....	.76	.87	3008
17..........	34.5	176.6	197.0	178.0	199.8	26	98.2	129.5	....	....	.90	.95	3061
18..........	22.8	158.3	185.4	159.6	189.0	34	169.8	107.8	....	....	.97	.93	3105
19..........	25.8	134.3	178.5	150.6	....	41	54.6	....	....	....	.66	....	3032
20..........	24.6	148.0	185.8	153.0	....	54	80.29	....	....	....	.88	....	3155

RESULTS OF THE EXPERIMENTS.

Table 2 gives the observed and calculated data from the tests. Curves 1 and 2 of Figure 11 also show graphically the relation of coefficients of heat transmission to air richness (Ps ÷ Pt) of the heating vapor. As heretofore stated, it was not possible to control conditions as carefully as was desired, the result being that, in most tests, there was more than one varying condition.

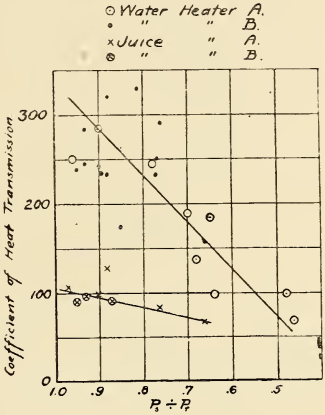

FIG. 11.

It was found to be impossible to control the removal of air through the vents satisfactorily. The methods used for venting have already been described on page 25. One of the causes of difficulty in this may have arisen from the fact that the condensation was removed at the "half-way" point of the steam path, whereas the vents were located at the end of the steam path. Although the condensation was removed through a sealing chamber, as heretofore described, this latter was often ineffective, the result being that vapors, and possibly air, were drawn out at the bottom. This would naturally interfere somewhat with the circulation of steam along the second half of its path. In other words, in order to get thorough vapor circulation in this part of the heater, and effective use of the heating surface, it would be necessary to remove the air at the end of the steam path. Perhaps a vacuum steam trap for removing condensation and a separate vacuum pump for removing air at the end of the steam path would have made possible more complete control of conditions.

Table 6 gives a summary of the coefficients of heat transmission obtained in the experiments on this apparatus.

TABLE 3.

COEFFICIENT OF HEAT TRANSMISSION.

		Max.	Min.	Average.
Heater A	Water	286	69	173
Heater B	Water	369	157	258
Heater A	Juice	134	67	98
Heater B	Juice	134	92	112

It will be noted that the coefficients obtained from heater A, which was connected to the second body, were considerably lower than those obtained from heater B, connected to the first body. This is doubtless mainly due to the fact that the removal of air was much more effective in heater B. The velocity of water through the tubes was 52 F. P. M. in the tests where water was used. Tests 1 to 12, inclusive, were made with water and tests 16 to 20 with juice. The heating tubes were supposed to be clean in all of these tests. The velocities named are much lower than is common for juice heaters. This was due to the fact that the heaters used were not originally designed for the experiments. 300 F. P. M. would have been a more desirable velocity.

Reference to Figure 11 shows that the coefficients obtained with juice were considerably lower than for water. Most of this difference is doubtless due to the effect of viscosity. Some of the difference, however, was probably due to the low velocity through the tubes, which, it will be noted, was little more than half that in the tests in which water was used.

By referring to Table 2 it will be noticed that the coefficients were determined in two ways, namely:

(1) Using steam temperature corresponding to the pressure (vacuum).

(2) Using steam temperatures as actually measured.

In the curves of Figure 11 the coefficients determined by the latter method were used.

The average increase of juice temperature in heater "B" was 31° and in heater "A" 17.7°, or, to put it in other words, the amount of heating done by the heater connected to the first body was $\frac{31}{17.7} = 175$ times that in the heater connected with the second body.

The experiments brought out very clearly the importance of thorough venting of air and drainage of condensation. The difficulties encountered in relation to these two points emphasize the advantages, especially as regards the capacity, of taking the heating vapors from other than the last body of a multiple effect, even though the last body has the advantage in the matter of economy. The average coefficients in these experiments, being obtained under unfavorable conditions, are low and therefore safe for use in designing heaters.

EXPERIMENTS ON THE VACUUM HEATERS AT CENTRALE FLORIDA.

Figure 12 is a diagram of the quadruple effect and vacuum juice heaters used in the experiments at Centrale Florida. Figures 13, 14 and 15 show the heaters in detail. M is the vapor pipe which conveys vapors from the first body of the quadruple effect to the vacuum heaters. Each heater comprises three tubular sections, one above the other. As shown, there are five of

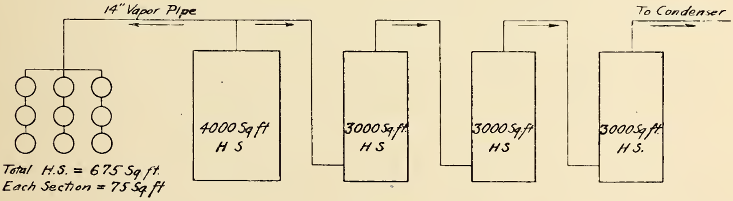

Fig. 12.

these units, A, B and C utilizing vapors for heating, whereas D and E utilize exhaust or live steam, the juice first passing through the vapor heaters. During the period of the experiments the vacuum heaters consumed vapor from the first body equal to about 10% on weight of cane. Figure 15 shows a sectional view of one of these sections, each section having ten tubes 2 inches O. D. and 15 feet long, making 75 sq. ft. of heating surface. The juice passes twice through each section, whereas the steam passes through once. This gives six juice passes and three steam passes for each unit. Juice enters at the bottom and leaves at the top. Steam or vapor enters at the top and leaves as condensation at the bottom. The total length of the juice path in one three-section unit is 90′ and the total length of vapor path 45′. Condensation is taken off at the end of the steam path. Juice is forced through the heaters and condensation removed from them by means of centrifugal pumps. As originally constructed no provision was made for removing air, a centrifugal pump being used for draining the condensation water. No air being removed, there was a gradual accumulation of air and gradually reduced

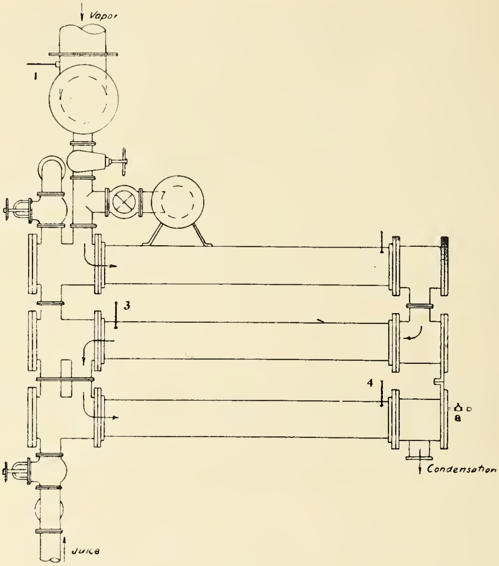

Fig. 13.

capacity during the operation of the heaters. To overcome this, ½ inch pipes were tapped into each of the three heater units at the end of the steam path at the points marked —a— and connections made to the condenser of the quadruple effect, globe valves being used for controlling the flow from each unit. Observations made during the tests showed that this arrangement proved effective in increasing the capacity of the heaters.

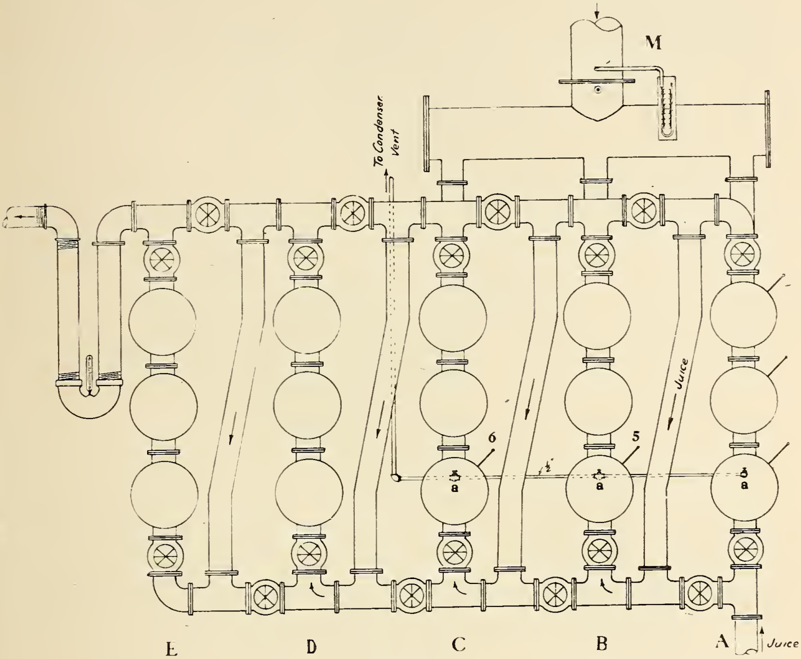

Fig. 14.

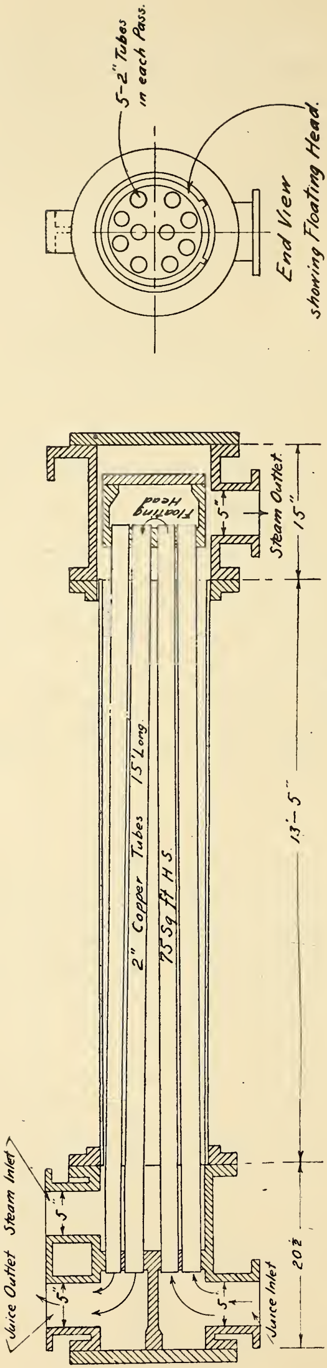
5-2" Tubes
in each Pass.
End View
showing Floating Head.
Floating
Head
5"
Steam Outlet.
15"
2" Copper Tubes 15' Long.
75 Sq Ft H S.
13'-5"
Steam Inlet
5"
Juice Outlet
5"
5"
Juice Inlet
20½"

FIG. 15.

SCOPE OF THE EXPERIMENTS.

The experiments on this apparatus were made for the purpose of securing data regarding the coefficients of heat transmission in regular operation with varying vapor pressure (vacuum) and varying air richness of the mixture. Special observations were made to determine the variation of air richness along the steam path.

PROCEDURE.

In making the tests, the general procedure was similar to that used in the experiments at Audubon Park, the juice being weighed on the regular juice scales of the factory. A running start and a running stop was used in each test. There was a juice feed tank between the scales and the heater, which made it necessary to measure the height of juice at the beginning and at the end of each test in order to make the proper correction. The thermometers were located at different places in the steam compartments shown on the drawing in direct contact with the steam. In some of the tests, all of these were read, while in others only a portion of them were read. The pressure (vacuum) of vapor entering the heater (the same as the boiling pressure in the first body) was observed by means of the mercury manometer near M. All thermometers used were carefully calibrated.

METHODS OF CALCULATING RESULTS.

The coefficients of heat transmission, the juice velocities and air richness, $\frac{P_s}{P_t}$ were calculated as for the tests at Audubon Park.

RESULTS OF THE EXPERIMENTS.

Two sets of experiments were made:

1. Tests of the general performance of the vacuum heaters.
2. Tests for the study of air in the vapor.

Table 4 gives the results of the former and Table 5 of the latter.

TABLE 4.

	Test Number			
	1	2	3	4
1. Duration of test, hours	2.66	1.33	4.29	3.00
2. Vacuum in vapor pipe to heater, inches	3.37	5.3	1.55	2.5
3. Temperature corresponding to the above Vacuum, °F.	206.2	202.4	209.5	207.8
4. Temperature of vapors entering heater, actual, (Ther. 1)	....	199.9	206.6	205.7
5. Temperature Vapor at end of Steam path. HEATER A	....	132.6	180.7	182.6
6. Temperature juice entering	81.7	82.2	85.3	86.0
7. Temperature juice leaving	118.0	185.7	206.2	194.8
8. Juice fed to heaters, lbs.	123,958	177,289	263,140	192,915
9. Velocity of juice, F. P. M.	121	346	159	167
10. Heating surface, sq. ft.	675	675	550	550
11. Mean temperature difference.	105.2	52.4	33.33	48.6
12. Coefficient of heat transmission	22.3	355.0	300.0	238.0
NOTE	No Vents	Vents		

TABLE 5.

	Test Number					
	5	6	7	8	9	10
1. Duration of test, hours	1.63	1.55	1.52	1.65	1.62	1.38
2. Vacuum in vapor pipe to heater	4.6	4.2	5.2	5.8	8.6	5.6
3. Temperature corresponding to the above vacuum	203.8	204.6	202.6	201.4	195.5	202.0
4. Temperature of vapors entering heater, (actual) (Ther. 1.)	203.7	203.0	201.0	200.8	194.4	200.8
5. Value of $\frac{Ps}{Pt}$	.996	.967	.965	.985	.976	.976
6. Temperature upper section Heater A (Ther. 2)	201.3	201.7	199.8	199.6	195.3	200.2
7. Value of $\frac{Ps}{Pt}$	.949	.942	.940	.963	.995	.965
8. Temperature middle section Heater A (Ther. 3)	172.4	196.5	191.4	197.1	155.8	197.1
9. Value of $\frac{Ps}{Pt}$	.507	.85	.79	.914	.41	.91
10. Temperature lower section Heater A (Ther. 4)	118.0	124.7	156.0	172.4	100.9	167.0
11. Value of $\frac{Ps}{Pt}$	.128	.15	.35	.52	.09	.47
12. Temperature juice entering Heater A	81.6	79.9	80.3	81.3	84.3	82.2
13. Temperature juice leaving Heater A	136.5	130.8	143.1	148.6	123.1	151.1
14. Temperature juice leaving Heater B	....	....	176.2	179.4	158.8	184.8
15. Temperature juice leaving Heater C	....	....	197.0	200.6	183.8	199.8
16. Juice fed to heaters, lbs	87,024	100,622	96,954	101,566	88,929	103,728
17. Velocity of juice, F. P. M.	138	168	165	160	142	195
18. Heating surface, sq. ft.	225	225	225	225	225	225
19. Mean temperature difference	92.1	96.2	87.2	81.9	90.40	80.60
20. Average coefficient of heat transmission	128.3	139.0	185.5	247.0	95.40	260.0

CONDITIONS PREVAILING DURING DIFFERENT TESTS.

(See Tables 4 and 5.)

Test 1—Heater operated according to the methods regularly employed in the factory. Condensation drained by centrifugal pump. This test made before vent connections (aaa) were provided (see Figure 14). Heating surface clean, the heater being a new one and operated for the first time 36 hours prior to these tests. The heater worked poorly, the steam compartment being relative cool, especially toward the end of the steam path.

Test 2—Same as Test 1, but made with vents (aaa) connected. Vent valves (½″) were partially opened to give higher temperature at end of steam path. 44 hours since heater started clean. This test a preliminary one.

Test 3—Practically the same as Test 2, but more reliable. 62 hours since heater started, clean.

Test 4—Same as Test 3, but only heaters A and B in operation, C being cut out. 65 hours since heater started, clean.

Test 5—Observations made on heater A only. Vent valve closed. 68 hours since heater started, clean.

Test 6—Same as Test 5, except that vent valve opened one-quarter turn. 80 hours since heater started, clean.

Test 7—Same as Tests 4 and 5, except that vent valve was opened ¾ turn. 84 hours since heater started, clean.

Test 8—Same as Test 7, except that vent was wide open (2½ turns). 88 hours since heater started, clean.

Test 9—Test 5 repeated. 100 hours since heater started, clean.

Test 10—Test 8 repeated. 104 hours since heater started clean.

Note.—The juice heated in these tests was limed cold to neutrality.

Referring to Table 4, it will be noted that the coefficient of heat transmission in Test 1 was only 22.3. The heater had been running since the factory started under this condition, the result being that most of the heating was done with exhaust and live steam in heaters D and E. This test was made with no provision for venting air, the centrifugal pump used for removing the condensation giving no assistance in this. After this test the vents (see Figures 13 and 15) were connected up and the remaining tests, 2, 3 and 4, made under conditions stated elsewhere. It will be noted that after this change the coefficients were much greater, the average for Tests 2, 3 and 4 being 290, with an average vacuum of 3.4″. It may be well to state here that these coefficients were calculated, using steam temperature due to the observed vacuum. The extra high coefficient in Test 2 was doubtless due to the high juice velocity. It will be noted that in these three tests there was an average temperature loss in the steam between its entrance to the heater and the end of the steam path of about 40°. The coefficients found in these tests may be considered relatively high, indicating good design and operation. The juice velocities, however, are somewhat below usual practice, it being common to design heaters for juice velocities around 300 F. P. M.

Table 5 gives data regarding Tests 5 to 10, inclusive, on heater A only. These tests were made principally for the purpose of studying the air conditions along the steam path and temperatures were measured at different points, as shown in Figure 13 along the steam path. The total length of steam path was 45′, the thermometers being placed about 15′ apart, namely, at the points 1, 2, 3 and 4. In these tests vent valve openings varied from closed to wide open, and the amount of air, $\frac{P_s}{P_t}$ was determined from the temperature measurements and the vacuum, it being assumed that the vacuum was the same in all portions of the steam compartment.

Table 6 gives the principal factors that might be expected to affect the coefficients obtained in the tests. The main factor, however, is the amount of air, $\frac{P_s}{P_t}$, the other factors being fairly uniform in all of the tests. The values of $\frac{P_s}{P_t}$ in the table are the averages obtained from the thermometer readings at the points 1, 2, 3 and 4. The average of the coefficients obtained in the tests with closed vents is 112, while that for the tests with vents wide open is 255.

TABLE 6.

Test No.	Average Coefficient of Heat Transmission	$\frac{P_s}{P_t}$	Velocity of Juice F. P. M.	Vacuum in Heaters	Vent Valve ½″ Open Turns
5	128.3	.645	138	4.6	0
6	139.0	.730	168	4.2	¼
7	185.5	.760	166	5.2	¾
8	247.0	.845	160	5.8	2½
9	95.4	.618	143	8.6	0
10	260.0	.830	195	5.6	2½

Figure 16 shows graphically the relation of air in steam $\frac{P_s}{P_t}$ length of steam travel. It will be noted that in the first 15′ there is little drop in the value of $\frac{P_s}{P_t}$, and it is difficult to

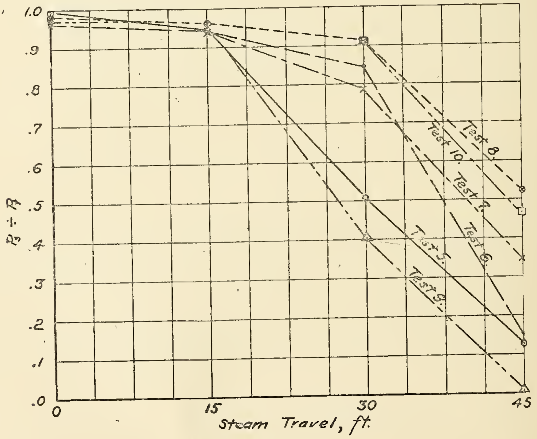

FIG. 16.

identify the curves. Beyond this point, however, there is a larger variation and identification of the curves for the different tests is easier. The curves ending with the highest values are those with the least amount of air in the steam and with the greatest vent openings. It will be noted that in some cases curves cross each other, showing erratic conditions in regard to the amount of air in the steam. The coefficients of heat transmission obtained in these tests and given in Table 6 are platted against $\frac{P_s}{P_t}$ in Figure 17. The points on this curve are quite regular

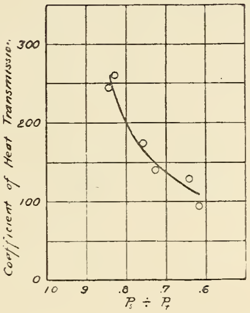

FIG. 17.

and each value of $\frac{P_s}{P_t}$ being the average of four other values, the curve may be considered as reliable. The two lowest points on this curve were obtained from tests made with the vent closed. The vent, however, had been closed for a limited time only, which accounts for the fact that the coefficients obtained were considerably higher than those obtained under similar conditions in Test 1, where the heater had been operating for many hours. Doubtless the value of $\frac{P_s}{P_t}$ might with proper design and operation be made still better than the best of those found in these tests. It is true that the method of venting used adds considerable to the work of the condenser and under certain conditions might tax it too much. Perhaps a vacuum pump might in some cases be more suitable for this work. A better separation of air might also be obtained by gradually decreasing the area of the steam path so that the air would be pushed in front of the steam, thus reducing the amount of steam vented with the air.

In Tests 7 to 10, inclusive, observations of the temperature of the juice leaving each of the heaters A, B and C were made. With these data it was possible to calculate the increase of temperature and the coefficient of heat transmission in each of these units. These values are given in Table 7.

TABLE 7.

Test No.	Coefficient of Heat Transmission			Increase of Juice Temperature		
	Heater A	Heater B	Heater C	Heater A	Heater B	Heater C
7	186.0	198.0	333.0	62.8	33.1	20.8
8	247.0	205.0	503.0	67.3	30.8	21.2
9	95.0	145.0	229.0	38.8	35.7	25.0
10	260.0	297.0	470.0	68.9	33.7	15.0

The curves of Figure 18 show the relation of juice temperature to juice travel based on the values in the table. If the temperature rise had been the same in each heater, the above relation would be shown by straight lines. An inspection of the

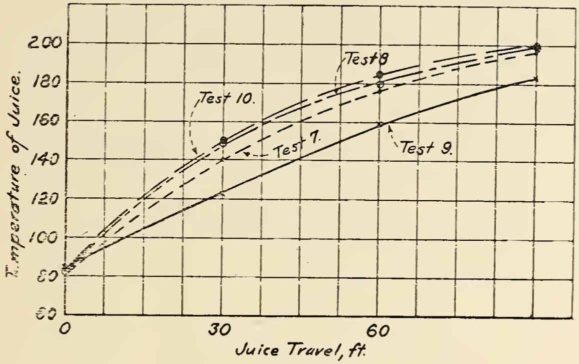

FIG. 18.

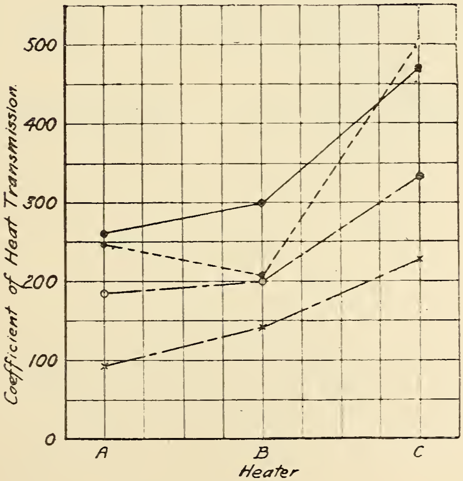

FIG. 19.

actual curves, however, shows that the temperature rise was decreased in each successive heater. This was, of course, partly due to the fact that the temperature fall is less in each succeeding heater, due to the increased juice temperature. Note that the curves of sharpest curvature correspond to those with the highest coefficients and lowest air content and vice versa. It will be noted also from this table that the coefficients were greatest in heater C. This is shown very plainly in the curves of Figure 19. It is probable that heaters B and C were vented more thoroughly than heater A for the reason that they were closer to the vent main (see Figure 12). It was often noticed during the tests that heaters B and C were hotter near the vents than heater A, this being demonstrated by placing the hand on the shell of the heaters at this point.

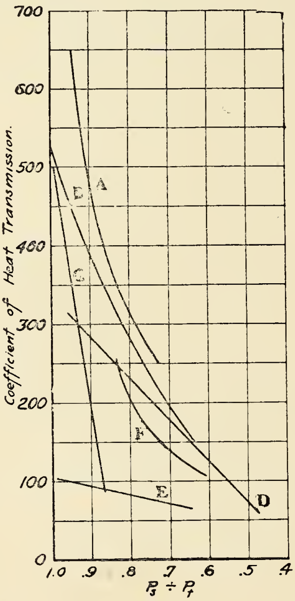

FIG. 20.

A—Louisiana Bulletin 149, page 23.
B—Louisiana Bulletin 149, page 23.
C—This Bulletin 149, Fig. 6.
D—This Bulletin 149, Fig. 11.
E—This Bulletin 149, Fig. 11.
F—This Bulletin 149, Fig. 17.

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Agricultural Experiment Station

OF THE

Louisiana State University and
A. & M. College,

BATON ROUGE.

TESTS OF BOILERS WITH BAGASSE AS FUEL

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Baton Rouge, La.
Ramires-Jones Printing Co.
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Tests of Boilers with Bagasse as Fuel

INTRODUCTION.

During the summer of 1909 the Louisiana State Experiment Station issued Bulletin 117 containing the results of a large number of tests made for the purpose of studying the conditions in different types of furnaces for burning bagasse with different modes of operation. These tests were made in Louisiana and Cuba during the grinding season of 1908. The conclusions derived from these tests were based mainly upon observations made upon the furnaces—the best furnace as far as heat economy was concerned having been assumed as that which delivered the greatest weight of gas to the boiler at the highest temperature from a given quantity of fuel. The observations included such items as weight of bagasse, moisture in bagasse, temperature of furnace, temperature of flue gases, draft in furnace and at base of stack, heat value of bagasse, composition of flue gases, grate area, and ratio of heating surface to grate surface. From these observations it was concluded that in a general way the best results were obtained in furnaces with high rates of combustion; with deep fuel bed over the entire grate, and with large combustion chambers lined with fire-brick. With these conditions, it was found that smaller quantities of excess air, higher furnace temperatures, and more complete combustion resulted. It was found also that where the ratio of heating surface to grate surface was low—in other words, where the boiler capacity was small—high stack temperatures resulted, indicating loss of heat at the stack. Many cases were found where the stack temperature ran as high as 700° Fahrenheit. From this it was concluded that many of the boiler plants tested were of a capacity too small for the best economy. In addition to recording the tests made, Bulletin 117 also contained a discussion of general principles and information regarding bagasse and bagasse furnaces, as well as the principles involved in calculating the results of the tests. In these tests the boiler was not considered except in so far as it directly affected the furnace. The water evaporated per pound of fuel is, of course, dependent not only upon conditions in the furnace but upon those in the boiler as well. It is evident that the cleanness of the heating surface, the circulation of the water, and the loading of the heating surface will all affect the proportion of the heat delivered by the furnace to that absorbed by the boiler and therefore the water evaporated per pound of fuel.

Having ascertained the general conditions existing in furnaces by means of the tests recorded in Bulletin 117, it was determined to make further tests such that the results could be measured in terms of pounds of water evaporated from and at 212° per pound of bagasse.

The object of this bulletin is to present the data obtained from several series of boiler tests made subsequent to those recorded in Bulletin 117. The object of these tests was to study the fuel economy of bagasse as affected by varying—

(1) The size and form of the combustion chamber.

(2) The air supply.

(3) The amount of overload.

(4) The size of grate, etc.

The tests recorded in the present bulletin have extended over several years and may be classified as follows:

(A) Tests at five factories during the grinding season of 1909.

(B) Tests during the grinding season of 1910 at Palo Alto Sugar Factory.

(C) Tests during the grinding season of 1914 at three factories; namely, Adeline, Vermilion Sugar Company, and Angola.

Two tests at Adeline made during the 1915 season are also included in this series.

BOILER TESTS, SERIES (A), 1909.

A total of twenty tests was made in five sugar factories, seventeen of which were on bagasse-burning boiler plants and the remaining three on oil-burning plants. In the following pages these five factories will be designated as a, b, c, d and e respectively.

Factory a, known as Poplar Grove, is the property of the Poplar Grove Planting Company, near Port Allen, La., and has one 300 H. P. Stirling boiler, with 3,000 square feet of heating surface for bagasse, and four 125 H. P. horizontal return tubular boilers for oil.

Factory b, known as Myrtle Grove, is the property of the J. Wilbert Sons' Planting & Manufacturing Company, and is located near Plaquemine, La. In this factory there were eight boilers of the horizontal return tubular type for bagasse. These boilers were in three batteries, one of which composed two boilers, each 6′x18′, with 70 4″ tubes, and a total heating surface of 3,092 square feet; another with two boilers, each 66″x19′ 6″, with 60 4″ tubes, and a total heating surface of 2,907 square feet, the remaining battery with four boilers, each 5′x20′, with 18 6″ tubes, and a total heating surface of 3,098 square feet. This makes a total of 9,097 square feet of heating surface, which at 12 square feet per horse power would give 758 horse power. As will be shown later, all of these boilers were used in only a portion of the tests.

Factory c, known as Waubun, was the property of the John T. Moore Planting Company, and is located at Shriever, La.

This factory has four horizontal return tubular boilers for bagasse, each having 1,260 square feet of heating surface, or a total of 5,050 square feet, making a total of 421 H. P.

Factory d, Southdown factory, is the property of the H. C. Minor estate, and is located near Houma, La.

This factory has, for bagasse, a battery of three boilers, each 5′x24′, with 20 6″ tubes; a battery of three boilers, each 6′x20′, with 70 4″ tubes, and a battery of three boilers, each 7′x20′, with 120 4″ tubes. This gives a total of 16,600 square feet of heating surface and a capacity of 1,383 H. P. For burning oil, only, there are also three horizontal return tubular boilers, each 5′x20′, with 52 4″ tubes, totaling 3,408 square feet of heating surface, or 284 H. P.

Factory e, Cinclare factory, is the property of H. L. Laws & Co., and is located at Cinclare, La.

This factory has five internally fired boilers, with Morrison corrugated suspension furnaces. Each of these boilers has a heating surface of 3,000 square feet, which at 10 square feet per horse power would be 300 H. P., or a total of 1,500 H. P. All of the boilers described above for bagasse have Dutch oven furnaces.

APPARATUS USED IN MAKING THE TESTS.

The feed water, in all tests except those at factory b, was measured in tanks of known volume and the weight calculated from the volume, with the proper temperature corrections. At factory b, a Worthington turbine meter was used for this purpose.

Fahrenheit mercurial thermometers, previously calibrated and reading to 212 degrees, were used for feed water temperatures. For flue gas temperatures a 1,000 degree mercurial thermometer was used.

A 3/8″ iron pipe, about 20″ in length and closed at one end, was filled with good cylinder oil and inserted into the stack with the open end outside. The thermometer was inserted into this bath of oil, which lessened the danger of breaking the thermometer and aided in getting average readings of temperature.

The temperature in the furnace was obtained by means of a Bristol portable pyrometer. The fire end consists of a compound-thermo electric couple.

For determining the flue draft, an ordinary U tube manometer was used. For the draft at the furnace, an Ellison differential draft gage, reading to hundredths of an inch, was used. For flue gas analysis an Orast apparatus was used. The amount of moisture in the steam was determined by means of a throttling calorimeter, made of pipe fittings. The sampling tube of the calorimeter was placed in the steam main near the boiler.

METHODS USED IN MAKING THE TEST.

The tests were, in all cases, conducted according to the code of the American Society of Mechanical Engineers. The method used for obtaining the weight of bagasse fed to the furnaces was to weigh the cane supplied to the mill, and to subtract from this the weight of juice; in other words, to determine the extraction in per cent of the weight of cane, and from this the weight of bagasse. In some of the tests all maceration at the mill was stopped; in others it was continued. The volume of the juice was measured in the clarifier tanks of the factory, and the density taken at frequent intervals, and from this the weight was calculated.

The cane was weighed by cars at the scale house. For starting a test a gap was made on the cane carrier just in front of the first car, and when this gap reached the mill the test was begun, the time being noted and simultaneous measurements of the feed water and the juice commenced.

Other observations taken regularly throughout each test, usually every thirty minutes, were temperatures of feed water, furnace, stack, and calorimeter; pressure in boiler; temperature of juice; the draft and flue gas samples in furnace and flue.

To determine the moisture in the bagasse, two or more oat sacks were filled about one-fourth full of wet bagasse and weighed. This was then placed on the top of the boiler setting to dry, and kept there until two successive weighings were the same. The original weight minus the last one gave the moisture. About three of these samples were taken for each test. On account of the large boiler plants handled and the lack of assistance, it was impossible to weigh the ash and refuse, so the same is not accounted for in the calculated results, as required in the A. S. M. E. Code. Former experiments, recorded in Bulletin 117, showed the percentage of ash to be quite uniform, so that for purpose of comparison the lack of these data may be considered practically negligible.

The tests were ended in much the same manner as the starting, a gap on the cane carrier being used for identifying the last weighed car of cane. The conditions maintained in the operation of the furnaces varied in the different tests in order to follow the scheme of tests decided upon at the beginning. These conditions will be considered for each factory separately.

(a) TESTS AT POPLAR GROVE FACTORY.

A total of six tests were made at this factory, three upon the 300 H. P. Stirling boiler, with bagasse as fuel, and three upon the horizontal return tubular boilers, with oil as fuel. Only the tests on the bagasse boiler will be considered here. This plant was grinding, at the time of the tests, at the rate of about 460 tons per twenty-four hours, which gave the 300 H. P. boiler a heavy overload. This condition gave opportunity for observing the effects due to overloaded heating surface and the object of the tests on this plant was mainly for this purpose. Another object of the tests was to secure data upon the effects due to varying the air supply by means other than by use of a stack damper, there being no stack damper.

The combustion chamber of the former was large and roomy. Air was supplied by a chimney and by a forced draft fan run from a steam engine. Feed water was supplied from a pump arranged especially for the tests drawing from a tank into which the water from two measuring tanks emptied alternately. The smoke stack and breeching connections were close.

The feed hoppers of the furnaces were of the counterweight flap door type, with no positive means for opening or closing. They evidently did little towards preventing air from flowing into the furnace. The flue gas samples and the flue temperature were taken at the base of the stack. The furnace temperature was observed at a point just back of the bridge wall, presumably the hottest part of the furnace. The setting of the boiler was in good condition as regards cracks, etc., so that the flue gas analyses may be taken as representing the products of combustion.

Conditions Maintained During the Different Tests.

Factory (a).

Three tests were made upon this boiler, the first of which was under the regular conditions maintained by those in charge at the factory. Test 1. This test gave the largest loading of the three, developing 467.2 H. P. The blower was used, the engine for same making approximately 90 R. P. M. (revolutions per minute). The ash pit doors were about three inches open.

Test 2. The principal change of conditions in this test was to reduce the load on the boiler as compared with that of the preceding test. This was done by hauling away something like one-third of the bagaasse in wagons.

With the reduced load the speed of the blower engine was reduced from 90 R. P. M. to 70 R. P. M. and the ash pit doors were kept entirely closed, as it was thought that, with the reduced feed and with no reduction in grate area, the air supply should be less in order to prevent a greater excess of air in test 2 than in test 1.

Test 3. The main object of this Test was to reduce the air excess in every possible manner. As there was no stack or flue damper, this had to be done at the front of the furnace. The ash pit doors were closed entirely and the hopper doors were operated by hand. By this means the doors were kept tightly closed for the minimum time necessary for dumping, giving conditions which it is likely could not be reached by any flap door hopper mechanically operated.

For each of the tests it was arranged to have cane from the same field, which was of the D.74 variety. The tubes of the boiler were clean and were blown off with steam a short time before each test was begun. Maceration was discontinued during all of the tests.

Results of the Tests.

Table 1 shows the results of the tests at this factory.

Table 1. Results of Tests at Poplar Grove, Factory (a).

		Test 1	Test 2	Test 3
1	Date of trial	Nov. 9	Nov. 10	Nov. 11
2	Duration of trial, hours	6.5	7.66	6.75
3	Weight of bagasse (moist) lbs	48,615	39,926	46,327
4	Per cent of moisture in bagasse	53.1	53.2	52.9
5	Weight of dry bagasse, lbs	22,970	18,725	21,820
6	Total grate surface, sq. ft	60	60	60
7	Total heating surface, sq. ft	3,000	3,000	3,000
9	Total water fed to boilers, lbs	90,721	85,179	85,190
10	Quality of steam, per cent dry	97	96.5	96.5
11	Water evaporated, lbs	87,998	82,208	82,207
12	Equivalent evaporation, F. & A., (from and at) 212°, lbs	104,783	97,957	98,028

Hourly Quantities

		Test 1	Test 2	Test 3
13	Bagasse consumed per hour, lbs	7,479	5,212	6,878
14	Dry bagasse consumed per hour, lbs	3,534	2,444	3,232
15	Bagasse, per sq. ft. grate surface, per hour, lbs	124.6	86.8	104.6
16	Dry bagasse, per sq. ft. grate surface, per hour, lbs	58.9	40.7	53.8
17	Equivalent evaporation, F. & A., 212°, per hour	16,120	12,788	14,523
18	Equivalent evaporation, F. & A., 212°, per sq. ft. of heating surface, per hour	5.05	4.00	4.55
19	Steam pressure, gage	86	84.3	86.4
20	Temperature of feed water, deg. Fahr	71	71	70
21	Temperature of flue gases, deg. Fahr	598	570	612
22	Temperature of furnace, deg. Fahr	1,626	1,670	1,604
23	Draft, inches of water:			
	Flue	.55	.55	.54
	Furnace	.22	.29	.35
24	Horse power developed	467.2	370.7	409.3
25	Builders' rated H. P	300	300	300
26	Per cent of builders' rated horse power developed	155.7	123.6	136.4

Economic Results.

		Test 1	Test 2	Test 3
27	Apparent evaporation, actual conditions, per lb. of fuel	1.81	2.06	1.84
28	Equivalent evaporation, F. & A., 212°, per lb. of fuel	2.01	2.46	2.12
29	Equivalent evaporation, F. & A., 212°, per lb. of dry bagasse, lbs	4.12	5.23	4.59
30	Per cent carbon dioxide in flue gases	13.36	12.5	13.7
31	Tons of cane ground during test	124.7	151.7	129.2
32	Kind of cane	D 74	D 74	D 74

Test 1.—Run with usual conditions. Ash pit doors open about 3 inches. Fan running. Heavy overload.

Test 2.—Ash pit doors closed. Blower speed reduced 20 R. P. M. Load reduced by hauling away about 30 per cent of the bagasse.

Test 3.—Air excluded in every possible way. Ash pit doors closed. Hoppers operated by hand.

DISCUSSION OF RESULTS AT FACTORY a.

The economic results are shown in item 28. In comparing tests 1 and 2 with large and small overloads we have equivalent evaporations of 2.01 pounds and 2.46 pounds respectively per pound of bagasse as fired; that is, an increase of 22.5 per cent with the lighter load.

By referring to item 4 it will be seen that the moisture in bagasse was about the same for the two tests, though test 2 was at a slight disadvantage as regards flue gas composition, since a smaller per cent of carbon dioxide means a large excess of air. This conditon existed even though the fan speed and the opening at the draft door were reduced. This was in all probability due to the fact that with the largely reduced feed the grates were not completely covered all the time, causing a greater quantity of air to flow into the furnace. This was also augmented by the greater furnace draft (see item 23) due to closing the draft doors, and which caused greater inflow of air at the hoppers, which, it will be remembered, were operating under the same conditions during these two tests.

In test 3, in which special precautions were taken to prevent air leakage at the hopper and at the ash pit doors, there was an equivalent evaporation of 2.12 pounds of water per pound of bagasse as fired. It will be noticed that the quantity of carbon dioxide (13.7 per cent) was only slightly larger than in the other tests, which goes to prove the contention of the writer that the only effective method of controlling air supply is by means of a stack damper or by the speed of the blower. The moisture in bagasse for this test was, it will be seen, appreciably more than in the other tests, so that the equivalent evaporation should be based on dry bagasse (item 29) rather than "bagasse as fired." This shows an evaporation of 4.59 pounds, against 4.12 pounds in test 1; that is, an increase of about 11.5 per cent. This, however, cannot be attributed to the change in air conditions alone. Although the load was intended to have been the same as for test 1, in reality it was considerably lower, as will be seen from item 24. A comparison of items 24 and 29 will reveal the fact that in the three tests the evaporation per pound of dry bagasse was practically inversely proportional to the overload. This would make it appear that the loading in test 3 was mainly responsible for the increased evaporation as compared with test 1.

b. TESTS AT MYRTLE GROVE FACTORY.

Four tests were made at this factory, the objects of which were practically the same as for factory a. A description of the boilers and their dimensions is given in Col. 2, Table 6. The boilers varied in capacity from 65 to 130 H. P. each, but the grate surface was 36 square feet for each.

The entire boiler plant was served by a single self-supporting steel stack, 150 feet high. There was no stack damper, but there were flue dampers with all except the two largest boilers. The bagasse was fed through hoppers and flap doors similar to those at factory a. The tubes in these boilers could not be cleaned each day, so it was thought best not to clean them during the tests in order to maintain as nearly equal conditions for all tests as possible. The condition of the heating surface may be assumed as fair, however, the factory having been running only a few days. The feed water was measured by means of a Worthington turbine water meter. The furnace temperatures were taken from one furnace in each of the batteries of two boilers each, holes having been made in the setting for inserting the fire end of the pyrometer. The flue temperatures were taken in the horizontal common flue leading to the stack. There was considerable leakage in the breeching between the boilers and the stack, which, no doubt, caused the stack thermometer readings to be lower than they should have been; also low carbon dioxide content in the flue gases. For this reason most of the samples were taken at the back connection of the boilers instead.

All of the boilers in this plant, except the two largest, were arranged with hollow blast grate bars and a forced draft outfit, though it had not been used since the erection of the large stack mentioned above. The factory was handling on an average of about 35 tons of mixed cane per hour, or at the rate of about 850 tons per 24 hours.

TABLE 2. RESULTS OF TESTS OF MYRTLE GROVE, FACTORY b.

		Test 1	Test 2	Test 3	Test 4
1	Date of trial	Nov. 16	Nov. 17	Nov. 18	Nov. 19
2	Duration of trial, hours	8.00	8.08	8.08	6.00
3	Weight of bagasse (moist) lbs.	127,515	134,396	115,036	96,972
4	Per ct. of moisture in bagasse	54.0	54.0	54.0	54.0
5	Weight of dry bagasse, lbs.	58,655	61,822	52,917	45,607
6	Total grate surface, sq. ft.	252	252	288	216
7	Total heating surface, sq. ft.	8,322	8,322	9,097	6,190
9	Total water fed to boilers, lbs.	231,799	224,021	241,794	170,630
10	Quality of steam, per ct. dry.	96.2	96.2	96.2	96.2
11	Water evaporated, lbs.	222,922	215,508	232,601	164,146
12	Equivalent evaporation, F. & A., 212°, lbs.	240,375	232,086	250,741	175,237

HOURLY QUANTITIES.

		Test 1	Test 2	Test 3	Test 4
13	Bagasse consumed per hr., lbs.	15,939	16,633	14,212	16,162
14	Dry bagasse consumed per hr., lbs.	7,362	7,651.5	6,550	7,601
15	Bagasse per sq. ft. grate surface, per hr., lbs.	63.3	66.0	49.4	74.8
16	Dry bagasse, sq. ft. grate surface, per hr., lbs.	28.8	30.4	23.8	35.3
17	Equivalent evaporation, F. & A., 212°, per hr.	30,047	28,723	31,033	29,206
18	Equivalent evaporation, F. & A., 212°, per sq. ft. of heating surface per hr.	3.61	3.45	3.41	4.56
19	Steam pressure, gage	92.3	87.6	89.3	88.9
20	Temp. of feed water, Fahr.	179	178	178	187
21	Temp. of flue gases, Fahr.	475	466	466	480
22	Temperature of furnace	1,355	1,462	1,491	1,370
23	Draft, inches of water:				
	Flue	0.7	0.78	0.86	0.90
	Furnace	0.085	0.085	0.044	0.07
24	Horse power developed	871	827	899.5	846.4
25	Builders' rated H. P.	694	694	758.0	675.8
26	Per cent of builders' rated H. P. developed	126	119	118.6	164.1

ECONOMIC RESULT.

		Test 1	Test 2	Test 3	Test 4
27	Apparent evaporation, actual conditions. per lb. of fuel.	1.75	1.61	2.02	1.69
28	Equivalent evaporation, F. & A., 212°, per lb. of fuel	1.88	1.72	2.179	1.81
29	Equivalent evaporation, F. & A., 212°, per lb. of dry bagasse, lbs.	4.09	3.75	4.74	3.62
30	Per cent carbon dioxide in flue gases	5.8	7.0	8.3	6.5
31	Tons of cane per 24 hrs.	870	853	845	860

Maceration of something like 12 per cent was kept up during the tests. The weight of maceration water used was determined by collecting the same in a sheet iron gutter at frequent intervals, running it into a barrel and weighing the amount for a given length of time. By averaging the flow per minute for these different determinations, a fairly accurate measurement was made. This was then used in calculating the extraction and the weight of bagasse.

Conditions Maintained During the Tests at Factory b.

The first two of the four tests were run with the usual conditions of the factory prevailing. One of the 65 H. P. boilers was not running, leaving a total of 694 H. P. in use for bagasse. The flue dampers, also the draft doors, were all wide open. This condition in connection with the large draft due to the high stack was evidently responsible for more air than was necessary, the furnace draft being 0.085 inch of water.

Test 3 was run with all the bagasse boilers in use, a total of 758 H. P. (rated), and the furnace draft was reduced to 0.044 inch of water, about half that in tests 1 and 2. The main object of test 3 was to observe the effect due to this reduction of the draft.

Test 4 was run with two 120 H. P. boilers cut out, making a total of 675.8 H. P. in use. The object of this test was mainly for observing the effects due to heavily loaded heating surface, there being an overload of about 65 per cent. The flue dampers were set to give 0.07 inch of water, preliminary experiments having shown this to be the smallest draft that could be used with the reduced grate area without choking the fires.

Discussions of Results at Factory b.

The most striking result revealed by these tests is that brought about by the flue damper manipulation in test 3, as shown by comparison of the figures in item 29. This is also very clearly shown by the fact that during test 3 considerably more horse power was developed, while, at the same time, much less cane was ground in a given time.

Although the carbon dioxide (item 30) partially shows the improved condition due to the damper manipulation in test 3, it by no means shows the actual condition, on account of the leaks already referred to. In order to determine the actual carbon dioxide content, some eight samples were taken from the back connections of the different boilers at different times during the test, the average of which was 14.1 per cent carbon dioxide. This shows that all the values in item 30 are too small, though they probably have approximately the proper relations one to the other.

The appearance of the furnace was greatly changed by the conditions in test 3. The flames, as observed from the back of the boiler, had a rolling motion and completely filled that part of the setting back of the bridge wall, whereas in the other tests, the velocity was evidently much greater and the flame was in the form of a thin sheet near the boiler shell. The closing of the damper also resulted in making the front of the boiler hotter.

Further figures upon the lowering of economy due to excessive overload are again shown in test 4, item 29.

c. TESTS AT WAUBAN FACTORY.

Three tests were made at this factory. Test 3 was made with the intention of largely decreasing the load by decreasing the speed of the mill and in this manner the quantity of bagasse, but at the end of the test it was discovered that practically the same tonnage had been ground per hour as in the other two tests. In working up this test it was also found that some error had been made in securing the data necessary for obtaining the extraction. For these reasons it was decided not to include it in the records. Tests 1 and 2 will therefore be discussed.

Table 3. Results of Tests at Wauban, Factory c.

		Test 1	Test 2
1	Date of trial	Nov. 23	Nov. 24
2	Duration of trial, hrs	8.	8.
3	Weight of bagasse (moist), lbs	73,196	71,642
4	Per cent of moisture in bagasse	54.0	54.0
5	Weight of dry bagasse, lbs	33,670	32,955
6	Total grate surface, sq. ft	100	100
7	Total heating surface, sq. ft	5,050	5,050
9	Total water fed to boilers, lbs	129,011	125,044
10	Quality of steam, per cent dry	98.8	98.8
11	Water evaporated, lbs	127,463	123,543
12	Equivalent evaporation, F. & A., 212°, lbs	140,621	132,671

Hourly Quantities.

		Test 1	Test 2
13	Bagasse consumed per hr., lbs	9,148	8,954
14	Dry bagasse consumed per hr., lbs	4,208	4,119
15	Bagasse per sq. ft. grate surface, per hr., lbs	91.5	89.53
16	Dry bagasse per sq. ft. grate surface, per hr., lbs	42.0	41.19
17	Equivalent evaporation, F. & A., 212°, per hr	17,577	16,583
18	Equivalent evaporation, F. & A., 212°, per sq. ft. of heating surface, per hr	3.48	3.28
19	Steam pressure, gage	80.8	64.0
20	Temperature of feed water, Fahr	148	176
21	Temperature of flue gases, Fahr	538	539
22	Temperature of furnace	1,462	1,560
23	Draft, inches of water:		
	Flue	.375	.27
	Furnace	.130	.10
24	Horse power developed	509.5	480.5
25	Builders' rated H. P.	421	421
26	Per cent of builders' rated horse power developed	121	115

Economic Result.

		Test 1	Test 2
27	Apparent evap., actual conditions, per lb. of fuel	1.74	1.72
28	Equivalent evap., F. & A., 212°, per lb of fuel	1.907	1.85
29	Equivalent evap., F. & A., 212°, per lb of dry bagasse, lbs	4.176	4.02
30	Per cent carbon dioxide gases	11.53	10.1
31	Tons of cane per 24 hours	499	478

There were four boilers in this plant, all of which were exactly alike, with Dutch ovens alike.

The factory was grinding at the rate of about 490 tons of cane per 24 hours during the tests, the boiler capacity, based on 12 square feet per horse power being 421 H. P. The flues were blown off with steam just before each test. The feed water was measured in a tank of known volume. The furnace temperatures were taken just back of the bridge wall in one of the outside boilers of the battery of four boilers. The flue gas temperatures and samples were taken in the main flue leading to the stack.

The flue connections were fairly close, so that the flue gas samples were probably fairly representative of combustion. The feed hopper doors here were mechanically operated and served their purpose well; in fact, better than we have usually found. Each boiler flue was equipped with a damper which was manipulated so as to give the minimum draft possible that would not choke the fire. It will be noted, however, that it was somewhat less for test 2 than for test 1.

DISCUSSION OF RESULTS AT FACTORY C.

The main discussion of this test will be given further on in this report, when it will be taken up in comparison with other furnaces of different designs and sizes. The two tests having been made with practically the same conditions prevailing, there is little room for comparison of results. It is interesting, however, to note that the evaporation per pound of bagasse (item 28) is almost the same for the two tests, a result which might reasonably have been expected. An inspection of other items shows the same condition of uniformity in the two tests.

d. TESTS AT SOUTHDOWN FACTORY.

A total of five tests was made, the object of which was, in the main, to get data upon the burning of bagasse and oil together in the same furnace.

It is, however, very difficult to get accurate data upon this subject, even from evaporation tests, because there is no way of measuring, separately, the amounts of water evaporated by the bagasse and by the oil.

The general method of attacking the problem in this case was to measure the weight of bagasse and the weight of oil, also the total water evaporated.

With these data it was hoped that by comparison with data from plants in which the two fuels were burned in separate furnaces, some idea could be gained as to the relative values of the two methods.

It is known from various tests on oil-burning furnaces that the fuel oil will easily evaporate 14.5 pounds from and at 212 degrees.

For the successful burning of fuel oil it is generally held that furnaces especially designed for the purpose should be used, the essential condition being to have the oil surrounded by a mass of white hot fire brick, in order to thoroughly vaporize it as it leaves the burner. It has been believed by a majority of sugar house engineers that a pile of green bagasse produces conditions exactly opposite to those stated above, and for this reason they have burned the oil and bagasse in separate furnaces. On the other hand, it may be said that a good bagasse burner produces very high temperature and if oil burners are so located as not to throw the oil directly upon the pile of bagasse there seems to be some reason for assuming that the high temperature due to the combined effect of the two fuels might be beneficial in maintaining the conditions necessary for economy.

Under very light loads—in other words, with a very light feed—oil burners are not economical on account of the fact that high temperatures are not maintained in the furnace. With this particular condition, especially, there is no doubt that burning it in a bagasse fire should add to economy. It was with a hope of throwing some light upon this matter that these tests were made. Incidentally, some attention was also given to the effect of overloading the heating surface.

This factory was grinding at the rate of about 1,070 tons of cane per 24 hours during the tests. The general practice of the factory is to burn all oil and bagasse together, in Dutch oven furnaces under boilers aggregating 1,393 H. P., based on 12 square feet per horse power. The factory also has three horizontal return tubular boilers, aggregating 278 H. P., which are fitted for burning oil only.

These boilers are kept as a reserve and are used during the summer for drying sugars, also during the grinding season while the house has suspended operation for cleaning.

Maceration was continued during the tests. The feed water was measured in tanks of known volume. The fuel oil used during the tests was measured in the underground oil tanks of the factory by determining the depth at the beginning and end of the test, and from this the volume and weight was calculated, the density having been determined with a spindle. Each of the three bagasse batteries had its own stack and the stack temperatures were measured at the base of each of these. Flue gas samples were taken at the back connections of the boilers as the breeching connections were too open for adequate accuracy. Furnace temperatures were taken from two of the nine boilers, as it was impossible to get access to any except the two on the ends. The temperatures recorded represent the average of the two readings.

None of the boilers were provided with flue or stack dampers. The draft doors were wide open at all times during the tests.

CONDITIONS MAINTAINED DURING THE DIFFERENT TESTS AT FACTORY d.

As in all plants where bagasse and oil are burned together, the number of oil burners in operation at any time depended upon the load being carried, there being times when, with very light load, all of them were closed.

Test 1 was made with the lightest load it was possible to get with the factory in operation. This was done on the day after a shutdown for cleaning, with no vacuum pans working. The load was carried by the bagasse boilers and was about 20 per cent less than the load generally carried.

Test 2 was made with the maximum load possible, the same boilers being in use and the same conditions prevailing as in test 1, the test being made at a time when the vacuum pans were all working.

Test 3 was made with about the same load as in test 2, but the battery of oil boilers was used in addition to those used in the previous tests. This was done in order to reduce the overload on the boilers and so that as little oil as possible could be burned with the bagasse. In fact, most of the oil was burned under the oil boilers, though it could not be arranged to prevent some having to be burned in the bagasse furnaces.

Test 4 was made on the bagasse boilers under conditions practically the same as in tests 1 and 2.

Test 5 was of short duration, and conducted under the same conditions as tests 1, 2, and 4. It has, therefore, been decided not to include it in the table of results.

The tubes of the bagasse boilers were thoroughly cleaned on the day preceding the first test, though it was not possible to blow them out again during the tests. According to the statement of the engineer of the factory, the heating surface of the oil boilers was not in good condition on account of scale accumulated during the previous summer run, which had not been entirely removed.

Discussion of Results at Factory d.

It will be seen that the equivalent evaporation per pound of oil has been assumed at 14.5. The total oil consumed during each test (item 8) was then multiplied by 14.5, and the product subtracted from the total equivalent water evaporated. The number thus obtained was taken as the equivalent water evaporated by the bagasse (see item 13), and this, divided by the weight of bagasse, gave the equivalent evaporation per pound of bagasse (items 29 and 30).

By reference to item 27 it will be seen that in all of the tests the boilers were considerably overloaded, the lightest being 14 per cent above normal. The flue gas composition also indicated unfavorable conditions as regards air supply.

A comparison of the figures in items 29 and 30 will show the economic conditions prevailing.

In test 3, where the conditions were nearest that of burning oil and bagasse in separate furnaces, the equivalent evaporation was 3.94 pounds per pound of dry bagasse. In tests 1, 2, and 4, in which the oil and bagasse were burned together, the evaporation was 3.112, 3.27, and 3.96 pounds respectively per pound of dry bagasse. It will be noted that these figures differ, though the conditions were practically the same. It is difficult to account for this lack of uniformity in results. The equivalent evaporation per pound of dry bagasse in test 3 is practically the same as that for test 4, yet the average for the three tests in which the two fuels were burned together is 3.44, which when compared with the 3.94 pounds for test 3 seems to indicate better economy when the fuels are burned separately. It should be remembered, too, that the oil-burning boilers used in test 3 were not in good condition, and it is reasonable to suppose that if they had been in prime condition the difference would have been greater.

An interesting fact brought out in these tests is that of the total horse power required to operate the factory, the average maximum being about 2,050 H. P. It should be stated, however, that this includes the work done by a closed feed water heater which was found, during the tests, to deliver the heat equivalent of about 100 H. P. to the feed water. This would therefore make the net boiler power about 1,950, or 1.8 horse power per ton of cane ground per 24 hours. This series of tests was the only one in which the total factory boiler plant was tested.

e. TESTS AT CINCLARE FACTORY.

The boilers at this factory are of a type comparatively little used for bagasse burning. They are of the internally fired type with Morrison suspension furnaces, the fuel being burned in a Dutch oven in front of each boiler. In this type of furnace, the hot gases, after leaving the Dutch oven, enter the large corrugated flues which are entirely surrounded by water, and then return through small tubes to the front and the stack.

The object of the tests was to obtain data regarding the performance of this type of boiler and furnace.

Two tests were made upon the bagasse boilers which aggregate 1,200 H. P., based upon 10 square feet of heating surface per horse power. The air for combustion is supplied by means of a chimney in connection with a blower which supplies air at a pressure of about 6 ounces per square inch. Air leakage at the hopper is reduced by introducing the bagasse between two rollers at each hopper, the rollers being held together by means of springs. The hollow blast grate bars are arranged so they can be oscillated for the purpose of cleaning them. This is undoubtedly a very good feature.

TABLE 4. RESULTS OF TESTS AT SOUTHDOWN FACTORY, d.

		Test 1	Test 2	Test 3	Test 4
1	Date of trial	Nov. 29	Nov. 30	Dec. 1	Dec. 2
2	Duration of trial, hours	8.5	8.0	7.9	8.0
3	Weight of bagasse (moist), lbs.	198,370	173,300	170,636	174,738
4	Per ct. of moisture in bagasse	54.3	54.3	54.3	54.3
5	Weight of dry bagasse, lbs.	90,646	79,198	77,980	78,855
6	Total grate surface, sq. ft.	272	272	272	272
7	Total heating surface, sq. ft.	16,660	16,660	20,004	16,660
8	Total oil consumed, lbs.	12,603	20,266	18,147	17,779
9	Equiv. evap., per lb of oil (assumed), lbs	14.5	14.5	14.5	14.5
10	Total water fed to boilers, lbs.	445,443	512,734	521,404	523,400
11	Quality of steam (assumed), per cent dry	97.0	97.0	97.0	97.0
12	Water evaporated, lbs.:				
	By oil	169,843	263,433	234,673	229,479
	By bagasse	262,327	233,918	271,088	278,218
13	Equivalent evaporation, F. & A., 212°, lbs:				
	Total	465,028	554,468	569,220	570,368
	By oil	182,751	293,868	263,135	257,795
	By bagasse	282,277	260,277	306,085	312,573

HOURLY QUANTITIES.

		Test 1	Test 2	Test 3	Test 4
14	Bagasse consumed per hr., lbs.	23,334	21,662	21,599	21,842
15	Dry bagasse consumed per hour, lbs	10,664	9,899	9,671	9,856
16	Bagasse per sq. ft. grate surface, per hour, lbs.	85.7	79.5	79.4	80.2
17	Dry bagasse per sq. ft. grate surface, per hour, lbs.	39.2	36.6	36.2	36.2
18	Equivalent evaporation, F. & A., 212°, per hour:				
	By oil	21,500	36,733	33,308	32,224
	By bagasse	33,209	32,575	38,618	39,071
19	Equiv. evap., F. & A., 212°, per sq. ft. heating surface, per hour:				
	By oil	1.23	2.21	2.00	1.52
	By bagasse	2.00	1.96	2.32	2.35
20	Steam pressure, gage	82.5	82.0	77.4	83.0
21	Temp. of feed water, deg.:				
	Upper tank	81	78	76	77
	Lower tank	190	152	143	146
22	Temp. of flue gases, Fahr.	532	594	526	588
23	Temperature of furnace	1,560	1,675	1,450	....
24	Draft, inches of water:				
	Flue	0.345	0.490	0.430	0.48
	Furnace	0.11	0.120	0.106	0.086
25	Horse power developed:				
	Oil	626.1	1,064.7	964.4	934.0
	Bagasse	962.6	944.2	1,119.3	1,132.5
	Total	1,588.7	2,008.9	2,083.7	2,066.5
26	Builders' rated H. P.	1,392	1,392.0	1,667	1,392
27	Per cent of builders' rated H. P. developed	114	144.2	124.9	147.6

ECONOMIC RESULT.

		Test 1	Test 2	Test 3	Test 4
28	Apparent evap., actual conditions, per lb. of fuel:				
	Oil	13.5	13.9	12.9	12.9
	Bagasse as fired....	1.33	1.35	1.59	1.59
29	Equiv. evap., from and at 212°, per lb. of fuel:				
	Oil (assumed)......	14.5	14.5	14.5	14.5
	Bagasse as fired....	1.278	1.342	1.61	1.78
30	Equiv. evap., F. & A., 212°, per lb. of dry bagasse, lbs.	3.112	3.27	3.94	3.96
31	Per cent carbon dioxide in flue gases...............	....	9.6	....	....
	Oil	....	....	7.85	....
	Bagasse...... ....,	....	....	8.00	....
32	Tons cane per 24 hours......	1,030	1,050	1,050	1,100
33	Ratio of weight of red cane to white cane............	2.1	4.5	4.0	1.3
34	Normal extraction...........	72.9	75.3	75.3	46.2

The feed water was measured with the apparatus used at factory a. The figures upon moisture in bagasse were obtained from the chemist of the factory, as were also those on extraction.

The second test was of short duration, on account of the breakage of a mill roller shaft which caused a shutdown of the factory. The figures therefore may not be very accurate. The moisture content also seems too low in this test, and for this reason the items based upon the weight of dry bagasse may be questioned. The factory was handling cane at the rate of about 1,150 tons per 24 hours, the cane being mixed, apparently about half red and half white.

CONDITIONS MAINTAINED DURING THE TESTS AT FACTORY e.

The regular running conditions of the boiler plant were maintained during the tests. The draft doors were wide open the entire time. The practice of the house is to draw and clean fires every six hours. A slight deviation was made from this, in that the fires were cleaned just before the tests were begun and were not cleaned again until after the test, with the exception of that which could be done by shaking the grate bars, making 8 hours between cleanings for test 1 instead of the usual six hours. In justice to the boilers it should be known that the heating surface was not clean at the time of the first test. This is especially true of the large corrugated flues, in the corrugations of which much soot and other matter accumulates which can only be cleaned when the boilers are cool. It was impossible to postpone this test until the boilers could be cooled off for this purpose. The fire was very low a great deal of the time in the furnace most distant from the mill; in fact, it seemed as though better results might have been obtained by cutting this boiler out. This was especially so of test 2.

TABLE 5. RESULTS OF TESTS AT CINCLARE FACTORY, e.

		Test 1	Test 2
1	Date of trial		
2	Duration of trial, hours	8.5	4.67
3	Weight of bagasse (moist), lbs	203,695	77,550
4	Per cent of moisture in bagasse	53.9	50.5
5	Weight of dry bagasse, lbs	93,822	39,163
6	Total grate surface, sq. ft	205	205
7	Total heating surface, sq. ft	13,200	13,200
8	Total water fed to boilers, lbs	345,257	128,758
9	Quality of steam, per cent dry	98.0	96.15
10	Water evaporation, lbs	338,353	126,612
11	Equivalent evaporation, F. & A., 212°, lbs	355,271	137,643

HOURLY QUANTITIES.

		Test 1	Test 2
12	Bagasse consumed per hour, lbs	23,964	16,620
13	Dry bagasse consumed per hour, lbs	11,038	8,393
14	Bagasse per sq. ft. grate surface, per hour, lbs	116.9	81.07
15	Dry bagasse per sq. ft. grate surface, per hour, lbs	53.8	41.43
16	Equivalent evaporation, F. & A., 212°, per hour	41,796	24,499
17	Equivalent evaporation, F. & A., 212°, per sq. ft. of heating surface, per hour	3.16	2.23
18	Steam pressure, gage	98	98
19	Temperature of feed water	182	150
20	Temperature of flue gases, Fahr	560	531
21	Temperature of furnace	....	....
22	Draft, inches water:		
	Flue	.20	.20
	Furnace	.09	.11
23	Horse power developed	1,211	860.5
24	Builders' rated H. P	1,200	1,200
25	Per cent of builders' rated H. P. developed	100.9	71.6

ECONOMIC RESULT.

		Test 1	Test 2
26	Apparent evaporation, actual conditions, per lb. fuel	1.81	1.66
27	Equivalent evaporation, F. & A., 212°, per lb. fuel	1.94	1.77
28	Equivalent evaporation, F. & A., 212°, per lb. of dry bagasse, lbs	3.78	3.51
29	Per cent carbon dioxide in flue gases	10.9	11.36
30	Tons of cane per 24 hours	....	....

DISCUSSION OF RESULTS AT FACTORY e.

In view of the unsatisfactory conditions explained above the equivalent evaporation figures, 3.78 pounds of water per pound of dry bagasse, cannot be taken as representing the normal performance of the boilers. This figure, when compared with like figures from preceding tests, will be found low.

TABLE 6. SUMMARY, TESTS OF 1909.

Test number	Factory	Furnace draft, inches of water	Bagasse per sq. ft. G. S., per hour, lbs.	Cu. ft. furnace vol. per sq. ft. G. S.	Dry bagasse per sq. ft. G. S., per hour, lbs.	Equiv. evap. per sq. ft. H. S. per hour, lbs.	Temp. flue gases, deg. F.	Temp. furnace, deg. F.	Flue draft, inches of water	H. P. developed.	Per cent rated H. P. developed.	Equiv. evap. per lb. bagasse as fired, lbs.	Equiv. evap. per lb. dry bagasse	Per cent carbon dioxide, flue gases	H. P. (bagasse) developed per ton of cane per 24 hrs.	CONDITIONS
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	a	.22	149.6	6.62	70.6	5.05	598	1626	.55	467	155.7	2.01	4.12	13.36	1.02	Regular running. Large overload.
2	a	.29	104.2	6.62	48.8	4.00	570	1670	.55	370	123.3	2.46	5.23	12.50	1.12	Load reduced 30 per cent. Part of bagasse hauled away.
3	a	.35	137.5	6.62	64.6	4.55	612	1604	.54	409	136.4	2.12	4.59	13.7	0.89	Load slightly reduced. Air supply reduced at hopper and draft doors closed.
4	b	.08	63.3	4.12	28.8	3.61	475	1355	.70	871	126.0	1.88	4.09	5.80	0.99	Regular running. Flue dampers open. Strong draft.
5	b	.08	66.0	4.12	30.4	3.45	466	1462	.78	827	119.0	1.72	3.75	7.0	0.97	Same as for test 4.
6	b	.04	49.4	5.38	23.8	3.41	466	1491	.86	899	118.6	2.18	4.74	8.3	1.07	Boiler power 9 per cent more than in tests 4 and 5. Furnace draft reduced half by closing dampers.
7	b	.07	74.8	5.23	35.3	4.56	480	1369	.90	846	164.1	1.81	3.62	6.5	0.98	Strong draft. Heavy overload.
8	c	.13	91.5	5.90	42.1	3.48	538	1462	.37	599	121.0	1.91	4.18	11.5	1.02	Regular running. Normal load.
9	c	.10	89.5	5.90	41.2	3.29	539	1560	.27	480	115.0	1.85	4.02	10.1	1.02	Same as for test 8.
10	d	.11	85.7	11.00	39.2	2.00	532	1560	.34	1588	114.0	1.43	3.12	.....	....	Lightest possible factory load. Oil and bagasse burning together.
11	d	.12	79.5	11.00	36.6	1.96	594	....	.49	2008	144.2	1.50	3.27	9.6	....	Heavy load. Oil and bagasse burning together. Bagasse boilers.
12	d	.12	79.4	11.00	36.2	2.32	526	1450	.43	2085	148.8	1.79	3.94	7.85	....	Heavy load. Part of oil burned under separate oil boilers.
13	d	.09	80.2	11.00	36.2	2.35	588	....	.48	2066	123.9	1.78	3.96	.....	....	Same as for tests 10 and 11.
14	e	.09	81.0	6.20	41.4	1.78	531	....	.20	860	77.3	1.88	3.51	11.36	1.04	Regular running.
15	e	.12	116.9	6.20	53.8	2.53	560	....	.20	1211	80.7	1.74	3.78	10.90	1.07	Regular running.

GENERAL RESULTS.

For convenience, the accompanying summarization table, containing the most important data, has been compiled from the different tests. In comparing the figures from the different furnaces it will be noticed that the best evaporation per pound of dry bagasse was made in the furnace (Poplar Grove) with the highest temperatures, also having the highest carbon dioxide content. Further comparison of the different furnaces burning bagasse only shows that the evaporation was, in a general way, proportional to furnace temperature and that the furnace temperatures were proportional to their respective rates of combustion. (See table below.) This is corroborative of similar results reached in a different manner recorded in Bulletin 117.

Factory	Av'g Furnace Temperature	Av'g Rate of Combustion	Av'g Evap. per lb. Dry Bagasse
A........................	1633	130	4.64
C........................	1511	90	4.10
B........................	1420	62	4.00

Factory d is not included in this table for the reason that oil and bagasse were burned. In discussing this subject it may be well to call attention to the fact that the bagasse at Factory a, where best results were obtained, came from a relatively small mill, without a crusher, the rolls being 5′-6″ long. The cane was there carefully placed on the carrier by hand. It should also be noticed that the cane was all of the D.74 variety at Factory a, while at the other factories tested there was a mixture of white and purple cane. This would go to show that, pound for pound, the bagasse from white cane is as good as that from purple cane. This is contrary to the general belief, which is probably based upon the fact that the white cane produces more juice than the purple varieties of cane and therefore does not produce so much bagasse, while at the same time there is more work to be done by the boilers, due to the greater quantity of juice to be evaporated.

In column 5 of table 6 is the "ratio of furnace volume to grate area," a quantity calculated for the purpose of comparing the volumes of the different furnaces. It is generally held by authorities that for burning very moist fuels the best results are obtained with furnaces of large volume. An inspection of these figures shows no consistent relation between fuel economy and furnace volume. This, however, was hardly expected, as other conditions differed widely in the different plants. An item of interest, however, may be noted in the fact that the ratio of furnace volume to grate area varied from a minimum of 4.12 at factory c to a maximum of 11 at factory d.

Data concerning the boiler horse power developed by the bagasse is given in column 16 of the summarization table. This quantity varied from a minimum of 0.89 to a maximum of 1.12, the average being 1.02. These results were obtained with the boilers considerably overloaded, excepting the tests made at Cinclare factory.

As has already been stated, the maximum total power developed by the boilers at Southdown factory, where oil and bagasse were burned together, was 1,950 horse power, or 1.8 horse power for every ton of cane ground per 24 hours.

SUMMARY OF RESULTS (1909 TESTS).

The tests showed that decreasing the load on heavily overloaded boilers resulted in increased economy. In these tests on one boiler, with overloads of 55.7 per cent, 26 per cent, and 23.6 per cent, there was an equivalent evaporation of 4.12, 4.59, and 5.23 pounds of water per pound of dry bagasse respectively.

It was found that little change in economy could be produced by attempts to reduce the air supply by closure of the ash pit doors or the fuel hopper doors. Regulation of the draft, however, by means of the stack damper, resulted in substantial increase of economy. An increase in evaporation of 26 per cent resulted from halving the furnace draft in a plant having a very high chimney. This was done by partially closing the flue dampers.

The tests furnish no data upon the question of furnace volume and its effects upon economy.

The best fuel economy was found in the furnaces having the highest temperatures.

The highest furnace temperatures were invariably in the furnaces having the highest rates of combustion and accompanied by the highest carbon dioxide content in flue gases.

The power developed by the boilers with bagasse as fuel varied from 0.89 to 1.12 horse power per ton of cane per 24 hours, the average being 1.02.

The total power developed in the plant burning oil and bagasse together was 1.8 horse power per ton of cane per 24 hours.

SERIES (B) 1910 PALO ALTO.

This series of tests was made at the Palo Alto sugar factory in connection with experiments made for the purpose of studying the question of bagasse drying. Some of the results of these tests have already appeared in Louisiana Bulletin 128, entitled ''Bagasse Drying.'' In Series (A), already described, the methods employed in making the boiler tests were not satisfactory in every respect. This was due to the fact that the tests had to be carried out upon the entire set of bagasse-burning boilers at each factory, giving little opportunity for controlling conditions. The methods employed for determining the weight of feed water were seldom very accurate also, volumetric measurements being resorted to in all cases.

In the tests at Palo Alto conditions were more favorable for controlling conditions. The tests were all made upon a Stirling boiler with 1,000 square feet of heating surface, which was set aside for the experimental work in view for the whole grinding season by the owners of the factory. This boiler was completely fitted as an experimental plant, a special feed water pump being provided.

Figure 1 shows the general construction and dimensions of the boiler and furnace. The area of the grate for most of the tests was 20 sq. ft.; that is, 4′ long 5′ wide, the bridge wall being placed over the back end of the Gordon Hollow Blast Bars. Toward the end of the season experiments were made with the bridge wall moved back to the position shown by dotted lines, thus giving a larger grate area. The bagasse, which had been previously weighed, was fed through the temporary hopper X shown by dotted lines, the regular hopper being closed at such times. The temporary hopper was also provided with a door so that it could be closed when tests were not being conducted. The furnace was arranged so that it could be operated with forced draft or natural draft at will, the hollow blast bars being connected up with a Sturtevant blower, the discharge pipe of which was provided with a gate. When operating with natural draft, the blower was not operated, but by keeping the blast gate open a considerable draft of air was obtained through the hollow bars. It will be noted that the top of the furnace ends in an arch back of the bridge

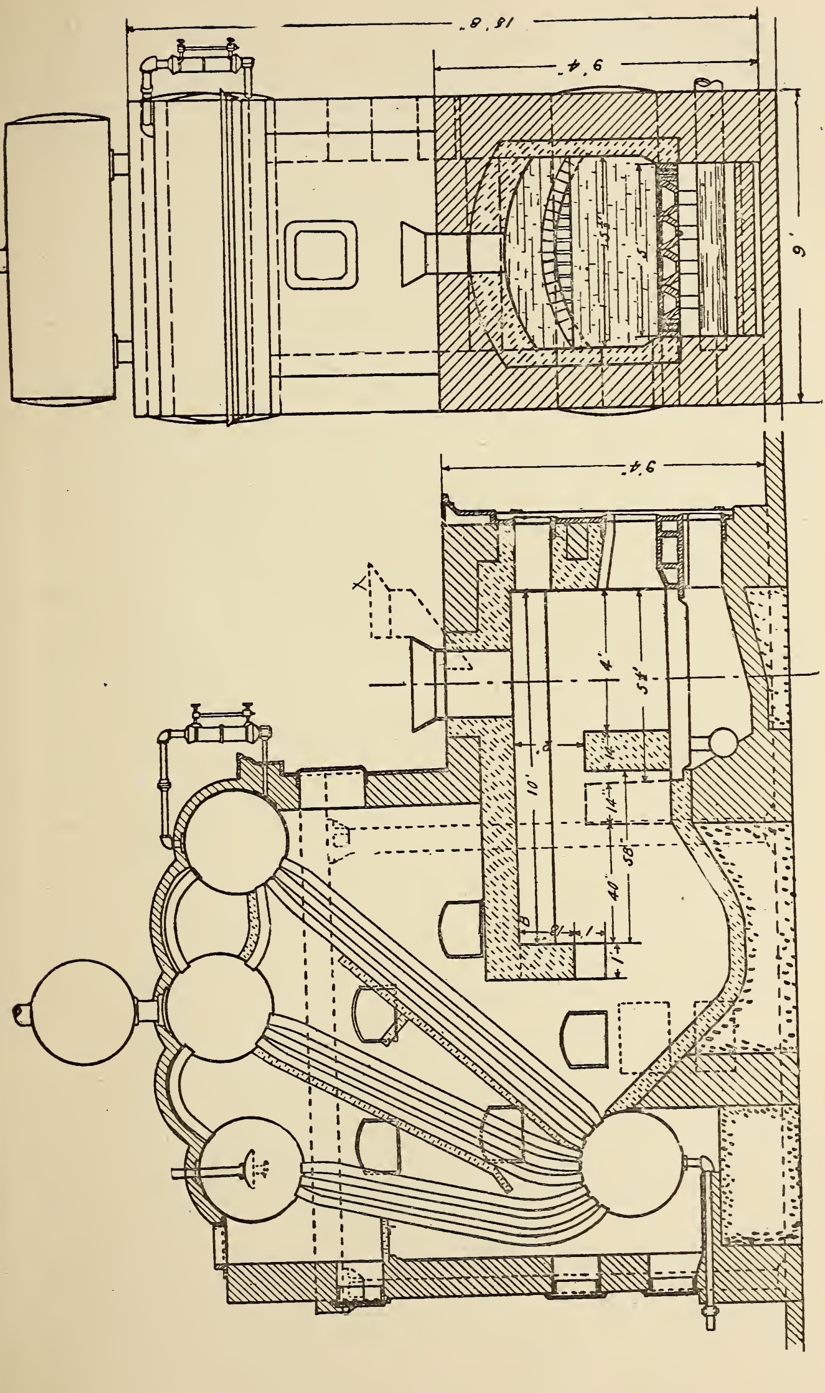

Fig. 1

wall. The object of this arch was twofold; namely, to bring about a more complete admixture of the gases just prior to their contact with the heating surface and to render the heating surface near the lower end of the tubes more effective by causing the flames to strike lower down.

APPARATUS USED IN MAKING THE TESTS.

A throttling calorimeter was used for determining the quality (dryness) of the steam leaving the boiler. The feed water for the experimental boiler was weighed automatically by means of a Wilcox water weigher which had been calibrated previously. Two Ellison differential draft gauges were used for determining the draft in the furnace and ash pit respectively. The flue gases were analyzed by means of an Orsat apparatus. For flue gas samples, a Hays gas sampling apparatus was used, by means of which an average sample was obtained. In this way only one gas analysis was obtained for each boiler test, although individual samples were made occasionally for the purpose of checking the average sample. Fahrenheit thermometers were used for determining the temperature of the gases, in the flue near the uptake from the boiler. Considerable attention was devoted to the determination of moisture in bagasse. The apparatus used consisted of nine or ten cracker cans of about one cubic foot capacity each, in which the samples of bagasse were collected and then placed upon the top of the boiler setting near the flue. The temperature at this place was sufficient to dry the samples thoroughly in about 24 hours. The tin cans were found to be better than sacks for the reason that the latter often catch on fire and burn. The weighing of the bagasse samples before and after drying was done with druggist scales in the laboratory of the factory.

METHODS USED IN MAKING THE TESTS.

The methods used in making the tests were practically the same as those already described for Series (A) except that the weight of feed water was determined more accurately, also the bagasse fed was actually weighed. In fact, the general arrangement of the plant and the possibility of controlling same facilitated experiments of a more exact nature.

Just prior to beginning each test, the blow-off valve of the boiler was opened for a short time and the boiler tubes blown off with steam. In this manner the boiler was made to operate under like conditions as regard cleanliness of the heating surface for all tests. The operation of the furnace was as near that of the regular methods used in the factory as possible, the fires being cleaned by the regular fireman about once every four hours and sliced every two hours. The draft doors were usually wide open. The blower used in the tests with forced draft supplied a pressure of about 1½″ of water in the blast pipe, just where it entered the setting of the boiler.

RESULTS OF THE TESTS.

In these tests there were five principal variables; namely, moisture in bagasse, rate of combustion, percent of rated capacity actually developed, draft and method of supplying draft; that is stack draft or blower draft. Of these, the last two only were under control during the tests. The principal results obtained in the tests are given in table 7. It will be noted that the moisture in bagasse varied considerably. This was due to the fact that the mill was small and its capacity scant for the work to be done. This made it necessary to run it under conditions that made it impossible to maintain uniformly high extraction and low moisture in bagasse with different kinds of cane. While there was some variation in the rate of combustion, the data secured were not of a nature to make possible any reliable conclusions regarding its effect upon efficiency. It will be noted that in most of the tests the horse power developed (see column 14) was somewhat less than the rated. In a few cases the load was very low indeed, which may account in part for the very low efficiencies obtained.

The relative economy in the different tests may best be compared by means of the unit "efficiency" (column 17) or "water evaporated per pound of dry bagasse" (column 16). That higher efficiency was obtained with low draft is very clearly shown. Four of the tests where natural draft was used with an average flue draft of .45″ of water show an average efficiency of 40.5 per cent. The other four tests with natural draft, having an average flue draft of .14, showed an average efficiency of 48.7 per cent. Doubtless the reduced efficiency with high flue draft was due to the increased amount of excess air, which is accompanied by low CO_2 in the flue gases. That this is the case is shown by the fact that in four tests with high flue draft the average CO_2 was 4.6 per cent, whereas with the low flue draft the average CO_2 was 6.3 per cent

Table 7. Summary, Tests of 1910.

Test number	Steam pressure, lbs. gage	Average Temperature, Fahr.			Average Draft, Inches Water			Flue Gas Analysis, %			Kind of draft	Moisture in bagasse, %	Rate of comb'n, lbs. of bagasse per sq. ft. of grate surface per hour	Per cent of rated H. P. developed	Water F. & A. 212° per lb. of bagasse as fired, lbs.	Water F. & A. 212° per lb. of dry bagasse, lbs.	Efficiency, furnace and boiler	Remarks
		Feed water	Flue	Furnace	Ash pit	Furnace	Flue	CO$_2$	O	CO								
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
3–	106.0	151.3	398.0	....	.16	.34	.44	3.8	17.0	0.0	Nat.	54.0	54.0	40.7	1.283	2.380	38.9	
4–	104.3	126.2	430.0	....	.0	.33	.50	4.1	16.1	0.3	Nat.	55.2	88.0	63.5	1.244	2.255	39.1	Strong draft. Fire dull during most of test. Bagasse feed somewhat light.
5–	112.0	137.0	435.0	....	.004	.143	.187	9.4	9.6	0.3	Bl.	53.5	89.0	90.6	1.755	3.280	52.7	Strong draft (Vacuum). Dull fire. Feed as heavy as was possible under conditions.
6–	109.0	153.0	442.0	1486	.012	.138	.159	7.9	12.4	0.1	Bl.	52.6	79.0	89.0	1.948	3.700	57.5	Better fire than in test 4, apparently low vacuum in furnace.
7–	106.0	155.7	404.0	1466	.016	.164	.162	6.3	14.0	0.4	Bl.	52.6	75.0	71.0	1.648	3.135	48.5	Furnace cold at start. Furnace very hot at end. Apparently fine fire.
13–	103.0	171.0	518.0	1475	.118	.350	.59	4.8	15.4	0.0	Nat.	55.5	96.0	83.0	1.484	2.675	47.3	Medium vacuum in furnace. Heavy feed.
14–	104.0	171.0	484.0	1550	.013	.250	.36	5.0	15.4	0.0	Nat.	55.5	93.0	80.5	1.500	2.700	48.0	High vacuum in furnace. Gases moved through furnace at high vel. with pulsating effect.
15–	105.0	171.0	453.0	....	.01	.180	.22	5.4	14.8	0.0	Nat.	56.2	86.0	76.5	1.533	2.732	48.5	Medium vacuum in furnace. Draft doors closed. Furnace full of bagasse.
22–	109.0	113.0	503.0	....	.048	.266	.483	8.4	11.6	0.2	Bl.	54.4	122.0	103	1.440	2.653	44.6	Combustion chamber very bright with whitish color. Med. vaccum in furnace.
23–	99.1	136.2	540.0	....	.015	.186	.250	6.3	11.1	0.6	Nat.	54.5	96.0	97.5	1.680	3.083	52.5	High rate of combustion. Vacuum low medium.
36–	109.4	123.5	489.0	....	.003	.123	.142	7.7	10.4	0.6	Nat.	56.0	62.0	88.0	1.793	3.230	56.6	Grate surface increased. Low vacuum.
37–	108.0	108.5	578.0	....	.026	.184	.324	5.2	12.8	0.2	Nat.	52.7	70.0	90.0	1.543	2.930	45.8	Enlarged grate area. Vacuum medium. Low moisture.
40–	109.3	179.7	552.0	....	.167	.29	.492	5.8	10.0	2.1	Nat.	51.8	74.0	75.0	1.275	2.462	36.8	Very high vacuum. Back arch removed.

The tests also show that this boiler operated with much higher efficiency when the blower was operated than when stack draft only was used. The average efficiency for the tests where blower draft was used was 51.5 per cent, whereas the average efficiency in the tests with natural draft was 45.1 per cent. This difference may also be expressed in terms of pounds of water evaporated from and at 212° per pound of dry bagasse, which for the tests with the blower was 3.16 and for the tests with natural draft 2.32. The reasons for this increase of efficiency with the use of forced draft are practically the same as for the increased efficiency with reduced draft. The boiler setting, although all possible precautions were taken to stop leaks, was somewhat open. The bagasse was fed through a hopper of a kind that made it impossible to prevent the inflow of a large amount of air when the draft (vacuum) in the furnace was high. Then, too, the grate was relatively large for the boiler, which made it difficult to feed the bagasse so that no portion of the grate would be uncovered. Naturally, the amount of air entering through such uncovered portions would be much greater with high draft than with low draft. The draft (vacuum) above the grate would, of course, be much less when the blower was operating. In fact, there was frequently some pressure as was shown by the fact that smoke and sparks were at times blown out through the feed hopper. Under such conditions, the inleakage of air and consequent stack loss added to excess air must have been relatively small. In calculating efficiency the heat delivered by the boiler in the steam was divided by the net heat supplied in the fuel. In order to show the method of calculating this item, let us assume a bagasse having a moisture content of 52 per cent and that by test each pound of bagasse caused the evaporation of 2.0 pounds of water F & A 212°. For each pound of bagasse there will be .48 pound of dry matter and .52 pound of water. The writer has found from various calorimeter tests of Louisiana bagasse, an average heat value of 8360 B. T. U. per pound of dry bagasse. The gross heat generated will, therefore, be $8360 \times .48 = 4012.8$ B. T. U. However, not all of this heat is available for producing steam in the boiler, some of it being required to convert the .52 pound of water into steam at the temperature of the stack, which temperature we will assume to be 500° F. The heat thus required will be .52 [212 — 70 + 966 + .5 (500 — 212)] = 651 B. T. U. The net heat theoretically available for steam production is therefore 4012.8 — 651 = 3361.8 B. T. U. per pound of bagasse as fired. The heat delivered by the boiler in the steam per pound of bagasse = 2 × 970.4 = 1940.8. Efficiency $= \frac{1940.8}{3361.8} = .576 = 57.6$ per cent.

That this particular boiler setting shows so much greater efficiency with low draft and with forced draft instead of natural draft, cannot be taken as evidence that such would be the case in all boiler plants. In fact, practice shows that very high efficiencies and perhaps greater efficiencies may be obtained with very high draft (vacuum) provided precautions are made to prevent inleakage of air, especially at the feed hopper and through cracks in the setting, and by carrying high rates of combustion; that is, by using small grates.

Table 8 gives a heat balance that has been calculated for the tests given in the preceding table.

TABLE 8. HEAT BALANCE.

1	2	3	4	5	6	7
Number of Test	B. T. U. per Pound of Bagasse as Fired	Per Cent Used in Evaporating Water in Boiler	Per Cent Loss in Dry Chimney Gases	Per Cent Loss Due to CO	Per Cent Loss Due to Moisture in Bagasse	Per Cent Radiation, Etc.
WET BAGASSE.						
3	3850	33.4	24.8	......	24.9	16.9
4	3758	32.2	22.3	7.2	25.9	12.6
5	3890	43.8	16.0	2.4	24.8	13.0
6	3920	48.3	11.2	1.4	24.35	14.75
7	3930	40.65	14.65	8.4	24.8	11.5
13	3730	38.5	28.0	......	26.3	7.2
14	3730	39.0	24.4	......	26.9	9.7
15	3775	39.5	20.2	......	26.5	13.8
22	3820	36.6	17.6	2.76	26.58	16.86
36	3780	46.0	13.8	9.5	26.7	4.0
37	3960	37.9	24.95	4.83	25.75	6.58
40	4035	30.7	15.6	3.68	25.0	....
Average	3848	38.88	19.46	5.02	25.7	11.5

In column 2 is given the heat value of dry matter in one pound of the bagasse fired, calculated from the calorimeter values. Of this heat supposedly generated, a portion is lost in the dry gases passing up the stack; a portion is lost in the steam generated from the initial moisture in the bagasse and from the moisture produced by the combination of the hydrogen and oxygen in the fuel and a portion may be lost due to incomplete combustion; that is, by burning the carbon to CO instead of CO_2 and the balance is lost in radiation and other small unknown causes. These different losses are given in columns 3 to 7, inclusive, of the table and are expressed as a percentage of the heat generated as given in column 2. One point which should be explained is the manner of arriving at the chemical composition of the fuel which is necessary in order to calculate the heat balance. These calculations assumed that carbon was the only element producing heat in the bagasse. The method of arriving at the amount of carbon is shown on page 12 of Louisiana Bulletin 117. Column 6 gives the loss due not only to the free moisture in the bagasse but that due to moisture produced by the combination of hydrogen and oxygen in the fuel as well.

SUMMARY—PALO ALTO TESTS.

With the type of bagasse furnace and setting used in these tests the boiler efficiency is lowered by excessive draft.

With this type of bagasse furnace and setting forced draft gives higher efficiency than stack draft. This is due to the fact that the blower causes reduced draft (vacuum) in the furnace and consequently less in leakage of air.

Bagasse furnaces and settings of this type should be provided with flue dampers so arranged that the draft (vacuum) can be positively and conveniently controlled.

As shown by the table of heat balances, the stack loss is very high, the average being $25.7 + 19.46 = 45.16\,\%$. The loss in the dry stack gases was high due to the large amount of excess air and the loss due to moisture was high due to the large amount of moisture in the bagasse and the moisture due to the combination of hydrogen and oxygen in the bagasse.

SERIES (C), 1914-'15.

This series of tests was made during the grinding season of 1914, with the exception of two tests in 1915 at Adeline. These tests were made for the purpose of securing data regarding the effect of rate of combustion, volume of combustion chamber, form of combustion chamber, methods of feeding bagasse and leakage of air into setting upon boiler efficiency or economy. In addition to the above, especial attention was given to the question of flue gas analysis.

The tests were made upon four typical bagasse boilers and settings, all of which represent the latest practice in bagasse-burning in Louisiana, all of them having been installed within the last three years. The experience gained in preceding experiments made it possible to conduct these tests along lines that would give results of increased reliability and usefulness. Consequently this series may be considered as the best yet made in these respects.

The general methods employed in carrying out the tests were much the same as employed in Series 1 and 2, except that the feed water was actually weighed on platform scales, whereas in former tests volumetric measurements were used. The bagasse fed during the tests was weighed in baskets holding approximately 25 pounds on platform scales. Especial care was taken in practically all tests to insure that the bagasse was uniformly and properly fed. In determining the amount of moisture in bagasse, average samples were obtained by taking a handful of bagasse at regular intervals from the baskets and placing same in covered buckets. At the end of each test a small average sample was obtained from the buckets, sealed in a closed can and sent to the University Laboratory, where the moisture determination was made in an electric oven.

TESTS AT ADELINE.

Tests 1, 2, and 4 were made on a boiler and setting shown in Figures 2 and 3. It will be noted that the combustion chamber is very large and elaborate in form. This type of furnace has been evolved in Hawaiian practice and this particular one designed by the Honolulu Iron Works Company. This boiler is one of a bat-

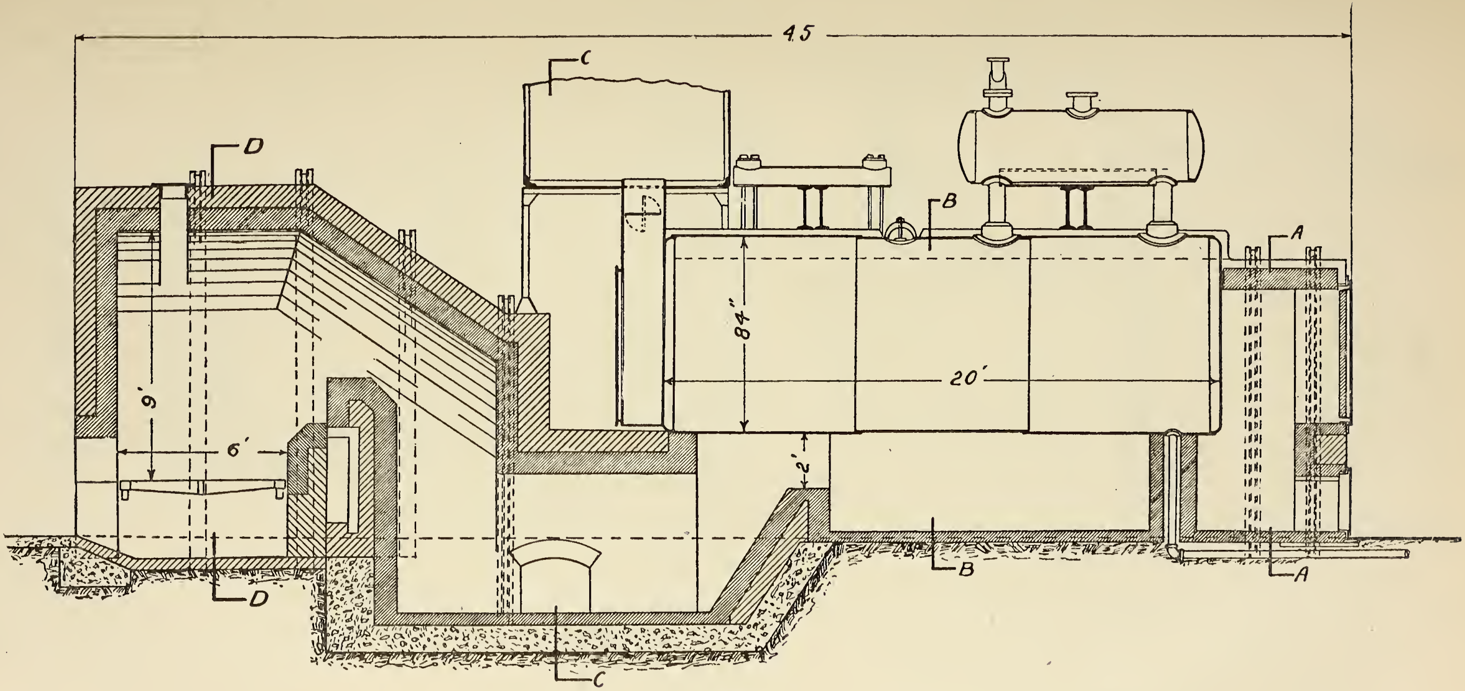

Fig. 2

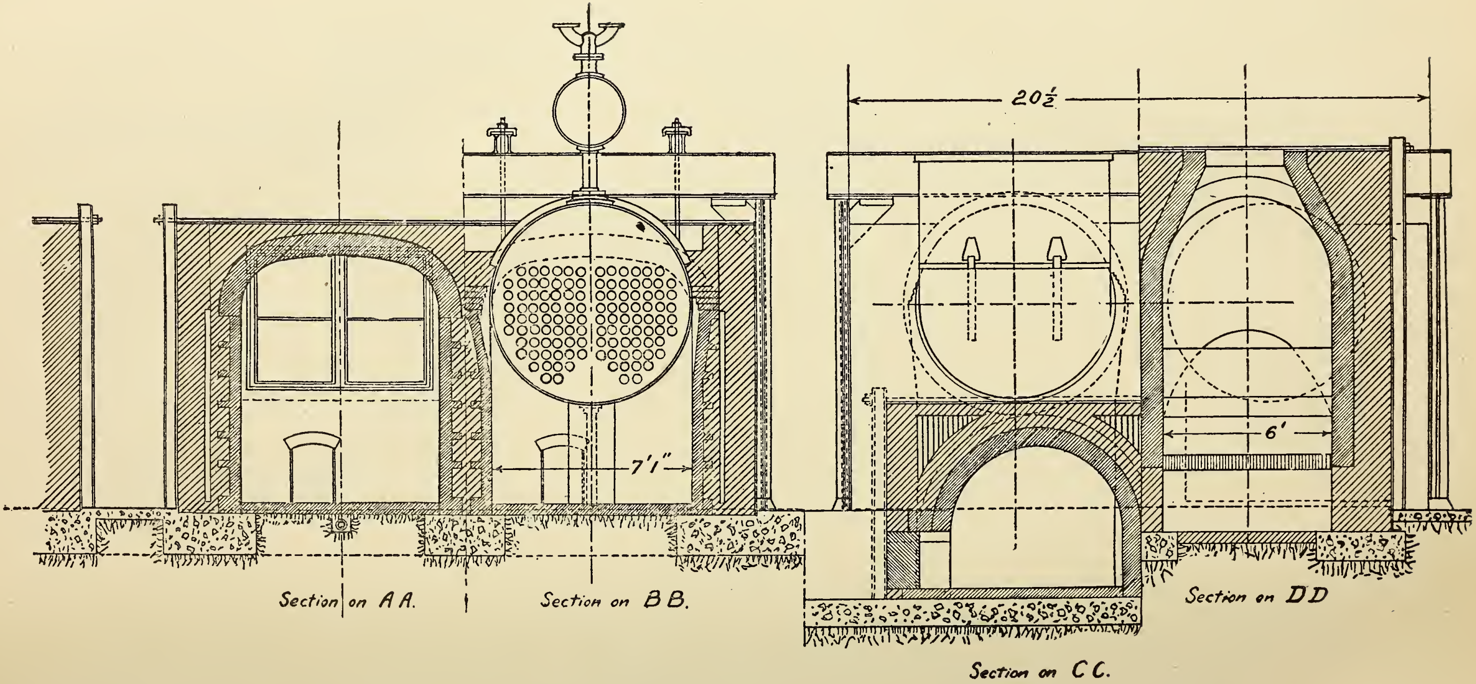
20½
7′1″
6′
Section on AA.
Section on BB.
Section on CC.
Section on DD

Fig. 3

tery or two, there being three batteries in the factory for burning bagasse, each boiler having a rated capacity of 250 H. P. Stack draft is supplied to the entire plant by means of a single concrete stack; the grate bars are of the ordinary Herringbone type, with ribs ¾″ wide and spaces ¾″ wide. The grate has an area of 25 sq. ft.; the bagasse is fed to the furnace by means of the belt rotary feeder manufactured by the Link Belt Company. There are dampers in the uptake flue of each boiler for regulating the draft. A special feed water pump was used for supplying the water fed to the boiler during the test. Test 3 was made on a small Dutch oven in the same factory. Before making the tests, the settings were gone over and the leaks stopped with cement as far as possible. The samples from which the flue gas analyses given in the tables were made were taken from the uptake flue. They may be considered as fairly representative of the products of combustion.

RESULTS OF THE TESTS.

Tests 1 and 2 were made in 1914 and Tests 3 and 4 in 1915. Tests 1, 2, and 4 were made upon the boiler shown in Figures 2 and 3 and test 3 on the small dutch oven. The conditions maintained in the different tests are given at the bottom of the table.

TABLE 9. (ADELINE).

TOTAL QUANTITIES.

		Test 1	Test 2	Test 3	Test 4
1	Duration of test, hours	8.0	8.0	8.0	7.85
2	Wt. of bagasse as fired, lbs.	44,912.2	30,222.6	32,359.0	34,666.0
3	Moisture in bagasse, per cent.	45.4	46.3	53.0	52.0
4	Wt. of dry bagasse, lbs.	24,522.1	16,229.5	17,150.0	18,026.0
5	Total grate surface, sq. ft.	25.0	25.0	25.0	25.0
6	Total heating surface, sq. ft.	2,500.0	2,500.0	2,500.0	2,500.0
7	Water evaporated, lbs.	96,144.5	67,234.5	65,927.0	74,198.0
8	Equiv. water evap., F. & A., 212°, lbs.	104,740.0	70,896.1	69,091.0	77,351.0

HOURLY QUANTITIES.

		Test 1	Test 2	Test 3	Test 4
9	Bagasse consumed, lbs.	5,614.0	3,777.8	4,044.9	4,416.0
10	Dry bagasse consumed, lbs.	3,065.3	2,028.6	2,147.5	2,296.4
11	Bagasse burned per sq. ft. grate surface, lbs.	224.6	151.1	161.8	176.6
12	Equiv. evap., F. A., 212°, lbs.	13,092.5	8,862.0	8,636.0	9,854.0

AVERAGE PRESSURES, TEMPERATURES, ETC.

		Test 1	Test 2	Test 3	Test 4
13	Steam pressure, lbs., abs.	118.9	118.6	96.7	100.1
14	Temp. feed water, Fahr.	167.3	194.5	201.4	205.5
15	Temperature flue gases	640.8	553.3	608.0	589.0
16	Temp. atmosphere outside	67.3	65.0	68.3	86.0
17	Draft, ash pit, inches water	.026	.012	.07	.05
18	Draft, furnace, inches water.	.182	.202	.23	.26
19	Draft, flue, inches water	.634	.464	.47	.53
20	Quality of steam	98.3	99.6	99.8	99.6

HORSE POWER.

		Test 1	Test 2	Test 3	Test 4
21	—H. P. developed	382.3	258.7	250.3	285.6
22	Builders' rated H. P.	250.0	250.0	250.0	250.0
23	H. P. developed, per cent of rated	151.8	102.7	100.1	114.2

ECONOMIC RESULTS.

		Test 1	Test 2	Test 3	Test 4
24	Equiv. evap., F. & A., 212°, per lb. of bagasse, lbs.	2.33	2.35	2.13	2.23
25	Ditto, per lb. of dry bagasse, lbs.	4.27	4.37	4.03	4.29
26	Efficiency (A) furnace and boiler	56.83	58.33	64.00	64.61
27	Efficiency (B) furnace and boiler	63.17	64.62	71.57	71.93

FLUE GAS ANALYSES.

		Test 1	Test 2	Test 3	Test 4
28	CO_2, per cent	9.80	11.90	10.20	13.50
29	O, per cent	9.40	7.90	8.00	5.70
30	CO, per cent	0.40	0.30	0.00	0.10

Conditions for Tests.

Test 1.—Boiler worked at heavy overload (about 50 per cent). Cane mixed, plant and stubble. Nearly all red. Draft doors wide open. Flue damper wide open. Fire cleaned just before start, also once during test, and sliced frequently. Tubes blown out with steam a few hours before start. Sixteen days since boiler was washed and cleaned.

Test 2.—Boiler worked at practically rated load. Flue damper partly closed. Draft doors partly closed. Other conditions about the same as in test 1.

Test 3.—Boiler worked at practically rated load. Draft doors open about 4 inches. Flue damper wide open. Other conditions as in previous tests.

Test 4.—Boiler worked at slight overload. Furnace equipped with a Thomas shaking grate which was shaken every fifteen minutes. Other conditions about as in test 3.

Discussion of Results.

In general it may be stated that the furnace shown in Figures 2 and 3 operated well. Attention has already been called to the large and elaborate combustion chamber with a large amount of refractory firebrick and many changes in direction of the products of combustion, the latter facilitating thorough mixing. As regards appearance, the combustion in this furnace seemed better than any of those tested. Evidently this type of furnace should be well adapted for taking care of variations in operation, particularly bagasse feed.

Tests 1 and 2 were made under conditions practically the same except for the load carried. Test 1 was operated at about 50% overload, while test 2 was made with practically rated load. The water evaporated per pound of dry bagasse—viz., 2.33 lbs. and 2.35 lbs., respectively—was practically the same in these two tests. This is indicated also by ''efficiency,'' item 26. Ordinarily, a boiler operating at large overload, other conditions being equal, will give a lower efficiency than when running at rated load. This, however, was evidently not the case in these tests. The setting offers an unusually large area of brickwork subject to leakage and radiation of heat, which losses would be practically the same whether the boiler is run at rated or overload. With overload, however, the percent loss would be smaller than with rated load. The reduced loss with overload probably offset the disadvantage due to overloading mentioned above.

It will be noted that two values for efficiency of furnace and boiler are given in the table (items 26 and 27). The efficiency (A) was determined in the manner described on page 34 of the Palo Alto tests; that is, by subtracting the heat required to vaporize the moisture in each pound of bagasse and raise its temperature to that of the stack from the gross heat value of a pound of wet bagasse and dividing same into the heat leaving in the steam generated by each pound of wet bagasse. In calculating efficiency (B) the same method was used except that the heat required to vaporize the moisture formed by the combination of hydrogen and oxygen in the fuel is also subtracted from the gross heat value of each pound of wet bagases, the latter method of calculating efficiency is probably more suitable for making comparisons where there is considerable variation in the quality of the bagasse.

It will be noted that flue gas analysis for test 2 was somewhat better than for test 1. This was probably due to the damper regulation in test 2. In other words, the wide open damper in test 1 gave more draft than was necessary for the best results.

Tests 3 and 4, with efficiencies of 64 and 64.6 percent respectively, seem to show no advantage for the large oven as far as economy is concerned. It is perhaps not proper, however, to take these two tests as conclusive in this respect. In both of the tests the bagasse feed was steady and there were no interruptions of service. With irregular feed the larger furnace would doubtless show up much better, the large furnace and refractory effect of much brickwork tending to produce better combustion under varying conditions. Tests 1 and 2 cannot be compared with tests 3 and 4 with any degree of certainty for the reason that the moisture in bagasse not only varied greatly but the method of determining the moisture was not the same in the two cases. Tests 1 and 2 were made during the 1914 grinding season, when the fibre was unusually high and hard, the result being that the moisture was unusually low, about 46%. As already stated, the moisture was determined in the University Laboratory. Tests 3 and 4 were made during the 1915 season, when the fibre was low and soft, resulting in high moisture in bagasse. In these tests, also, the moisture determinations were made in the laboratory at the sugar factory. Evidently there is some probability that the moisture determinations were not equally accurate. Moreover, since the moisture content enters into the calculation of efficiency it would not be proper to make comparisons of tests made during the two different years. For this reason, the tests cannot be depended upon to give reliable comparisons of the efficacy of the shaking grates used in test 4 as compared with the ordinary grates used in the other tests. It will be noted, however, that in test 4 the flue gas analysis was better than in any of the other tests, the CO_2 content being 13.5 per cent. It is very probable that this was due to the use of the shaking grates, which were shaken every fifteen minutes. It was noticed that the ash pit was always bright during this test, which was not the case with the other tests, showing that the shaking grate aided in securing a uniform supply and distribution of air through the fuel. It may be noted also that less cleaning and slicing of the fire was required during this test.

TESTS AT VERMILION SUGAR FACTORY.

The tests at this factory were made on the boiler and setting shown in Figure 4. The furnace is known as the Quinn Flat Top Furnace, the top being supported by I beams and special shaped bricks instead of an arch. The boiler and setting were designed and installed by the John H. Murphy Iron Works Company. The entire setting is encased in sheet steel with a lining of asbestos between the brick-work and the lining. The boiler was one of a battery of two of 250 H. P. each. The draft for the entire boiler plant is supplied by a single steel self-supporting stack, the grate bars are of the Herringbone type, the ribs being ¾″ wide and the spaces between ribs ¾″ wide. The bagasse is fed to the furnace by means of a Smith & Smith rotary feeder somewhat similar to that of the Link Belt Company at Adeline. There was a damper in the flue for regulating the draft. As at Adeline, a special feed water pump was used for feeding water to the boiler during the tests.

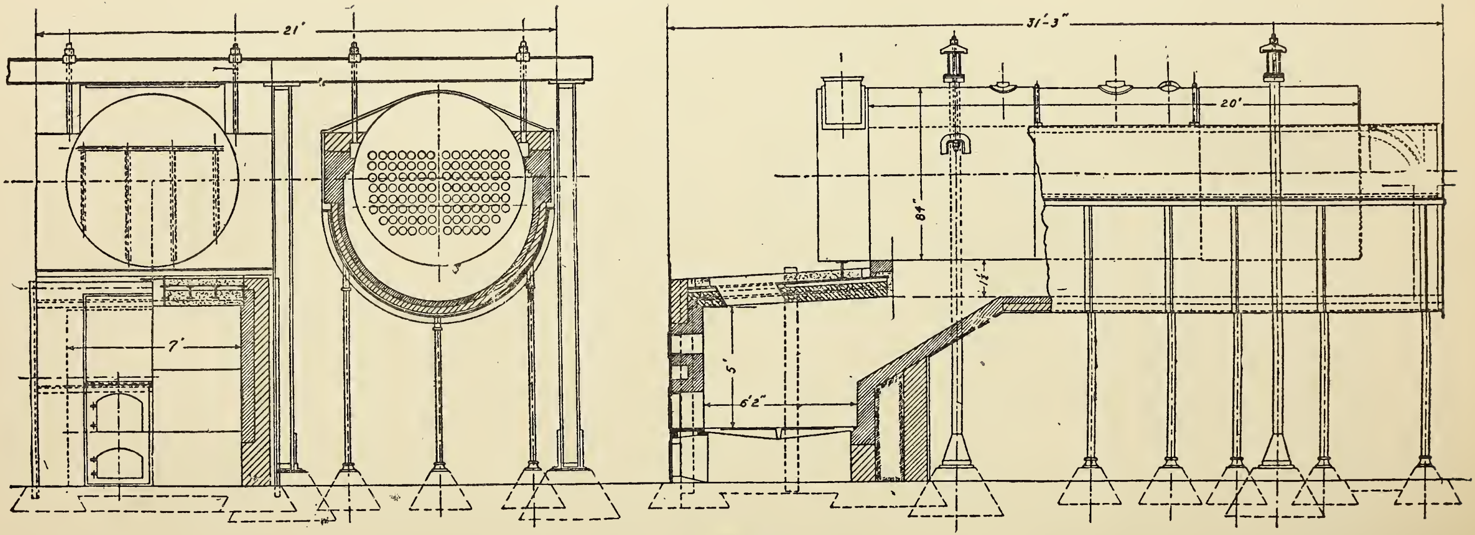

Fig. 4

RESULTS OF TESTS AT VERMILION SUGAR FACTORY.

The results of the two tests made at this factory are given in Table 10. The conditions of operation were practically the same for both of these tests, the second test being made mainly as a check. As will be noted, a slight overload was carried in each test. A somewhat thick fuel bed was carried in these tests, usually as high as the top of the fire doors. Both tests started with clean grate and the fire was sliced about every two hours during the tests. The draft doors were about half open or less. The bagasse burned in these tests was unusually dry for Louisiana conditions for the reasons explained for the Adeline tests. The conditions of operation were very favorable in both tests.

Since the conditions were practically the same in both tests, there is little room for comparison, except with tests on boilers at other factories. It will be noted that the efficiency was higher than in tests 1 and 2 at Adeline. The combustion chamber in this setting was smaller than in the large Adeline setting; also the grate was larger and the rate of combustion smaller than at Adeline. Both of these conditions would place the Vermilion plant at some disadvantage as compared with the Adeline plant. On the other hand, the Vermilion setting was very tight against inleakage of air, also well insulated as regards heat radiation. The former would tend to reduce the excess air loss, and the latter the radiation loss. It is probable that the reduced losses named offset the supposed disadvantages of small combustion chamber and low rate of combustion. It is well to keep in mind also the fact that one of the main advantages of large combustion chambers is to insure uniformly good combustion with variations in the conditions of operation. Since the conditions of operation at both Adeline and Vermilion were uniformly favorable, the Adeline setting had no opportunity to show its superiority in this respect. The flue gas analyses at Vermilion may be considered very good. That for test 2 has not only a low CO_2 content, but rather too much CO.

TABLE 10. VERMILION.

TOTAL QUANTITIES.

		Test 1	Test 2
1	Duration of test, hours	7.3	7.13
2	Weight of bagasse as fired, lbs	26,177.8	26,288.2
3	Moisture in bagasse, per cent	46.3	47.0
4	Weight of dry bagasse, lbs	13,887.3	14,182.5
5	Total grate surface, sq, ft	42.0	42.0
6	Total heating surface, sq. ft	2,450.0	2,450.0
7	Water evaporated, lbs	58,482.0	55,123.0
8	Equivalent water evaporated, F. & A., 212°, lbs	69,125.7	65,133.3

HOURLY QUANTITIES.

		Test 1	Test 2
9	Bagasse, lbs	3,586.0	3,687.0
10	Dry bagasse, lbs	1,902.4	1,988.3
11	Bagasse burned per sq. ft. grate surface, lbs	85.4	87.8
12	Equivalent evaporation, F. & A., 212°, lbs	9,469.3	9,131.3

AVERAGE PRESSURES, TEMPERATURES, ETC.

		Test 1	Test 2
13	Steam pressure, lbs., abs	109.8	108.7
14	Temperature feed water, Fahr	60.0	61.3
15	Temperature flue gases	529.0	529.0
16	Temperature atmosphere outside	56.1	61.0
17	Draft, ash pit, inches water	.014	.052
18	Draft, furnace, inches water	.155	.226
19	Draft, flue, inches water	.434	.417
20	Quality of steam, % dry	98.5	98.7

HORSE POWER.

		Test 1	Test 2
21	H. P. developed	276.5	266.6
22	Builders' rated H. P	250.0	250.0
23	H. P. developed, per cent of rated	109.8	105.9

ECONOMIC RESULTS.

		Test 1	Test 2
24	Equivalent evaporation, F. & A., 212°, per lb of bagasse, lbs	2.64	2.48
25	Ditto, per lb. of dry bagasse, lbs	4.98	4.59
26	Efficiency (A) furnace and boiler	66.65	61.15
27	Efficiency (B) furnace and boiler	73.81	67.65

FLUE GAS ANALYSIS

		Test 1	Test 2
28	CO_2, per cent	10.7	10.9
29	O, per cent	8.7	8.5
30	CO, per cent	0.0	0.3

TESTS AT ANGOLA.

The boiler and setting used in these tests is illustrated by Figure 5. This arrangement is similar to many others commonly found in Louisiana; in fact, it comes nearest the standard type of Dutch oven in common use. The top of the oven is supported by an arch. This outfit was designed and installed by the Payne & Joubert Machinery & Foundry Company. It is one of a battery of two of 150 H. P. each. Draft is supplied for the entire boiler plant by a single steel self-supporting stack. A blower is attached to some of the boilers, including the one tested, though its capacity is very small and the amount of air delivered by the blower during the tests was probably negligible. The grate bars are of the Herringbone type with ribs ¾″ wide and the spaces between ribs ¾″ wide. The feed hoppers are of the counterweighted flap type. Bagasse was fed into the hoppers, a basketful at a time, the flap being held closed between times. The draft was regulated by means of a damper in the uptake from the boiler. The draft was somewhat weak because the boiler tested was farthest from the stack, also a larger damper in the flue between the test boiler and the stack obstructed the draft in some of the early tests. This was discovered and remedied for the later tests. The quality of the bagasse in the tests at Angola was very variable; in fact, the general conditions of operation were less steady than at the other two factories. Due to this, it was though best to make a larger number of tests than at the other factories.

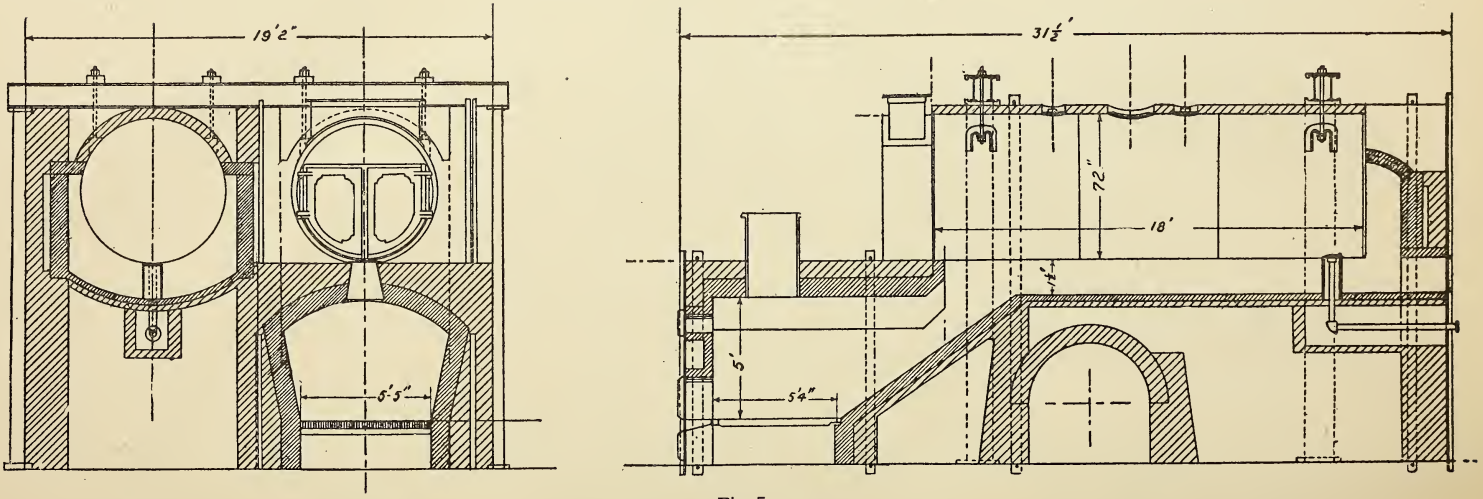

Fig. 5

TABLE 11. ANGOLA.

	TOTAL QUANTITIES	Test 1	Test 2	Test 3	Test 4	Test 5	Test 6	Test 7
1	Duration of test, hours	8.0	5.62	7.00	8.0	8.32	5.68	7.0
2	Weight of bagasse as fired, lbs	18,760.4	12,920.0	22,916.4	20,972.6	24,363.0	19,543.1	22,980.6
3	Moisture in bagasse, per cent	58.4	58.6	53.60	55.6	53.6	54.4	53.1
4	Weight of dry bagasse, lbs	7,804.3	5,348.9	10,226.6	9,311.8	11,304.4	8,921.4	10,789.4
5	Total grate surface, sq. ft	28.89	28.89	28.89	28.89	28.89	28.89	28.89
6	Total heating surface, sq. ft	1,512.0	1,512.0	1,512.0	1,512.0	1,512.0	1,512.0	1,512.0
7	Water evaporated, lbs	23,496.5	18,143.3	36,680.0	36,500.6	40,689.0	34,566.0	40,616.0
8	Equivalent water evaporated, F. & A., 212°, lbs	25,573.6	19,812.5	39,805.0	38,927.9	44,444.6	36,802.4	43,743.4
	HOURLY QUANTITIES							
9	Bagasse consumed, lbs	2,345.1	2,298.9	3,145.2	2,621.6	2,928.2	3,440.7	3,282.9
10	Dry bagasse consumed, lbs	975.5	952.3	1,460.9	1,164.0	1,359.2	1,569.8	1,541.3
11	Bagasse burned per sq. ft. grate surface, lbs	81.2	79.6	108.9	90.7	101.4	119.0	113.6
12	Equivalent evaporation, F. & A., 212°, lbs	2,196.7	3,527.2	5,686.4	4,866.0	5,343.8	6,475.9	6,249.1
	AVERAGE PRESSURES, TEMPERATURES, ETC.							
13	Steam pressure, lbs., abs	87.8	101.2	104.0	99.1	105.7	103.8	105.1
14	Temperature feed water, Fahr	146.3	144.8	162.1	168.9	151.9	176.5	163.1
15	Temperature flue gases	615.0	615.0	742.9	608.5	579.1	585.5	621.7
16	Temperature atmosphere outside	33.1	37.3	44.0	53.0	53.3	46.8	50.6
17	Draft, ash pit, inches water	.024	.034	.026	.157	.037	.017	.001
18	Draft, furnace, inches water	.170	.188	.128	.132	.212	.177	.144
19	Draft, flue, inches water	.374	.379	.338	.300	.487	.452	.386
20	Quality of steam	98.5	98.5	99.6	98.4	99.2	98.9	98.8
	HORSE POWER							
21	H. P. developed	93.3	102.2	166.0	142.1	156.0	189.1	182.5
22	Builders' rated H. P.	150.0	150.0	150.0	150.0	150.0	150.0	150.0
23	H. P. developed, per cent of rated	61.8	68.7	109.2	94.0	103.3	125.1	120.8
	ECONOMIC RESULTS							
24	Equiv. evaporation, F. & A., 212°, per lb. of bagasse. lbs.	1.36	1.53	1.81	1.86	1.82	1.89	1.90
25	Ditto, per lb. of dry bagasse, lbs	3.28	3.70	3.89	4.18	3.93	4.13	4.05
26	Efficiency (A) furnace and boiler	48.96	55.43	55.83	60.32	55.56	58.78	57.21
27	Efficiency (B) furnace and boiler	55.26	62.57	63.01	67.63	62.08	65.80	64.06
	FLUE GAS ANALYSES							
28	CO_2, per cent	9.80	11.90	10.20	13.50	9.1	9.25	11.2
29	O, per cent	9.40	7.90	8.00	5.70	11.1	10.75	9.4
30	CO, per cent	0.40	0.30	0.00	0.10	0.0	0.0	0.0

RESULTS OF THE TESTS AT ANGOLA.

The complete data from these tests are given in Table 11. However, for convenience in studying and comparing the results, the main factors which might be expected to affect the results are given in Table 12 with the conditions for each test below it.

TABLE 12.

No. of Test	1	2	3	4	5	6	7	Average
Moisture in bagasse, per cent	58.4	58.6	53.6	55.6	53.6	54.4	53.1	55.3
Rate of combustion	81	80	109	91	101	119	114	99.4
Temperature flue	615	615	743	608	579	585	622	623.9
Draft flue	.37	.38	.34	.300	.49	....	.45	.39
Per cent of rated load	61.8	68.7	109.2	94	103.3	125.1	121	97.6
Efficiency (A)	48.9	55.4	55.8	60.3	55.6	58.8	57.2	56.0
Per cent CO_2	2.5	7.3	9.8	8.7	9.1	9.25	11.2	8.3

Test 1.—Small load. Irregular bagasse feed; low draft. One-half of the grate bars hollow. Very moist bagasse.

Test 2.—Small load. Regular bagasse feed. Low draft. One-half of the grate bars hollow. Very moist bagasse.

Test 3.—Slight overload, regular bagasse feed. One-half of grate bars hollow. Relatively low moisture in bagasse.

Test 4.—Slight underload, regular bagasse feed. One-half of grate bars hollow.

Test 5.—About rated load. Low moisture. Regular feed. Grate bars all Herringbone. Draft stronger.

Test 6.—Considerable overload. Regular feed, strong draft. Herringbone grate bars. Moisture low.

Test 7.—Considerable overload. Regular feed, draft low.

NOTE.—The tubes in all boilers of this factory were blown with steam every six hours. The boiler was also washed out previous to the tests.

Test 1 was conducted with the conditions of operation exactly according to the usual methods in the factory. The bagasse feed was very irregular. The supply of bagasse to the furnace was regulated by means of a sliding gate, which was difficult to move; also difficult of access. The result was that at times the feed was very heavy and at other times very light. This resulted in a very cold furnace with portions of the grate uncovered at times and a very hot, though smoldering, fire at other times. That the grate was uncovered a large part of the time may be judged from the flue gas analysis, the CO_2 content being only 2.5 per cent.

In test 2 the conditions were practically the same as in test 1, except that the bagasse feed was regular. The increase of efficiency from 48.9 per cent to 55.4 per cent speaks for itself and illustrates the value of careful feeding and the need of arranging the furnace so that manipulation of the feeding apparatus can be done with convenience. A walk should be arranged along the front of the furnaces so that the fire tender can get to the feeder easily. A better arrangement is to have the feeders arranged so that they can be operated by the fire tender from the ground floor, in which case he can inspect the fires also. It will be noted that in both of these tests the bagasse was very moist. The cane had been wind-rowed for a long time and there had been much rainy weather previous to the tests, the result being that much of the cane was rotten, which caused very poor mill work at times.

The effect of high rates of combustion in causing better flue gas analyses is shown by these tests. Tests 1, 2, and 4, with rates of combustion less than 100, gave an average rate of combustion of 84, with a corresponding CO_2 content of 6.2 and boiler efficiency of 54.9. Tests 3, 6, and 7, with rates greater than 100, gave an average rate of combustion of 114, with a corresponding CO_2 content of 10.1 and boiler efficiency 57.3. It will be noted that in the latter three tests the boilers were considerably overloaded. This unfavorable condition, however, was overbalanced by the better combustion attending the increased rates of combustion.

The flue gas temperature was high in all of the tests, average 624. As the boilers were not overloaded (average 97.6 of rated), this would seem to indicate fouled heating surface. If so, it is probable that there must have been scale on the water side of the tubes, as inspection showed the tubes to be free of soot.

The average efficiency (55.3) was not bad for the conditions; in fact, as will be shown later, it compares well with the results obtained in the Adeline and Vermilion tests.

As is well known, it may be expected in a general way that higher efficiencies may be expected when a boiler is run at near rated load than when greatly overloaded, or greatly underloaded. When a boiler is run at underload the losses due to excess air, radiation, etc., are likely to be a greater proportion of the total. When a boiler is greatly overloaded the stack loss is likely to be high. The tests at Angola show that, of the two, overload is much to be preferred for bagasse-burning.

GENERAL DISCUSSION OF RESULTS, (All Tests.)

VOLUME AND FORM OF COMBUSTION SPACE.

The setting (Figure 2) of the boiler tested at Adeline was the only setting tested that had a combustion space of unusual size and form. The tests on this boiler, when compared with the tests on other boilers having smaller and simpler combustion chambers, show no particular advantage for the Adeline setting. For example, higher efficiencies were obtained with the Vermilion boiler than with the Adeline boiler, the former having a much smaller combustion space. This, however, may not be conclusive, not only because the Vermilion boiler had the advantage of reduced radiation and leakage losses, but because uniform fire conditions were maintained in both. The advantage of such settings as that at Adeline should be most pronounced under conditions of irregular feed and careless operation.

The volume and form of furnace rightly hold an important place in bagasse-burning. On account of the large amount of moisture in the bagasse and the resulting large volume of steam formed, a relatively large combustion space is required, else the high gas velocities will result in lack of time for complete combustion. There has probably been more variation in practice as regards this matter than in boiler furnaces for any other type of fuel, the fact being that there is no form of furnace that can really be called "standard." The same may be said of furnace volume.

TABLE 13.

	Cu. Ft. Combustion Chamber	B. H. P.	Cu. Ft. per B. H. P.	Cu. Ft. per B. H. P., Dutch Oven Only
Flue boiler with grate under boiler........	2,258.5	128	17.6	3.9
Dutch oven with H. R. T. boiler............	4,826.3	300	16.1	3.1
Dunn & Sutcliffe furnace with H. R. T....	3,963.0	200	19.8	5.9
Dutch oven with Stirling boiler............	8,796.0	150	5.8	1.5
Internally fired boiler, Dutch oven........	470.7	250	1.88	0.8
Bee Hive oven with Climax boiler..........	895.0	600	1.49	1.0
Dutch oven, H. R. T. boiler, Angola........	575.0	150	3.83	1.7
Quinn flat top, H. R. T. boiler, Vermilion..	646.0	250	2.58	1.2
Extended Dutch oven, H. R. T., Adeline...	1,500.0	250	6.00	2.5

Table 13 gives data regarding the volume of combustion space for a number of different types of settings and boilers investigated. One object of the Dutch oven is to insure complete combustion of the gases before they strike the relatively cool heating surface of the boiler. In view of this, it is somewhat difficult to determine just what should be designated as combustion space, especially in the case of H. R. T. and internally fired boilers. In column 4 of Table 13 the volume for all except the Stirling boiler includes the entire space between the grate and the back end of the tubes. Since only about one-eighth of the heating surface of H. R. T. boilers is in the shell, that portion of the setting which is under the shell may perhaps be consistently considered as combustion space. According to this view, the old flue boilers with the grate under the front end, also the Dunn & Sutcliffe furnace, give much greater combustion space than any of the others. These two types, as is well known, are practically obsolete. The last six types given in the table are of modern construction. Of these it will be noted that the Stirling boiler with Dutch oven and the H. R. T. boiler with extended Dutch oven used at Adeline give the greatest volume. In this connection it is well to note that, due to limitation of height of furnace and length of grate bars, the volume of combustion space per H. P. for a given type is likely to be less for large than for small boilers.

Large combustion volume also means a large amount of fire brick, which becomes incandescent and serves to intensify temperatures and to store heat which is given out when the fire is low, thus insuring uniformly good combustion. On the other hand, very large combustion chambers increase the radiation loss and the stack loss by increased leakage of air through cracks in the setting and through the pores of the masonry. The percent loss due to radiation is difficult to determine even in carefully conducted boiler tests, because of the difficulty of separating it from other small losses. It will depend upon the difference in temperatures of the two sides of the walls, the thickness of the walls, the amount of uncovered, exposed iron surface, the size of the boiler, the presence of air currents, etc. The radiation loss in coal-burning boilers probably varies from about 2 to 6 or 7 per cent of the heat in the fuel. With the increased size of furnaces used for bagasse-burning, it is naturally greater than for coal. In view of these considerations, it is not surprising that engineers differ widely as to the size and form of combustion chamber.

RATE OF COMBUSTION.

AREA OF GRATE.

Perhaps the most important question in designing bagasse furnaces is the size of grate, which depends upon the rate of combustion. It is the belief of many engineers that the excess air loss may be reduced by decreasing the size of the grate; that is, by increasing the rate of combustion, thus reducing the danger of excessive flow of air through the grate and fuel, especially where fire-room labor is such as to prevent the exercise of careful fire tending. On the other hand, excessive draft, which is required where high rates of combustion are maintained, tends to increase the air excess. The rate of combustion in practice varies from 50 to 300 pounds of bagasse per square foot of grate surface per hour, corresponding to 3⅓ B. H. P. to 20 B. H. P. per square foot of grate.

A study of the results of the tests recorded in this bulletin seems to show quite conclusively that the highest efficiencies were obtained with high rates of combustion, though it does not show the limiting values. In studying this question, the writer has made use of a large number of boiler tests, including not only those given in this bulletin but those of other experimenters as well, for the purpose of determining the general effect of rate of combustion on efficiency. The results of 31 boiler tests are shown in Table 14. All of these tests were carefully made on boilers representing good practice in Louisiana, Porto Rico, and Cuba. These figures show that as far as boiler efficiency is concerned (see second column) we cannot be assured that very high rates of combustion are necessary. It is true these tests are taken at random and represent different methods of operation and different designs of furnaces, though the general import of the figures may be given some weight. A large number of boilers recently installed in the tropics operate with very high rates of combustion, it being common to see 500 H. P. boilers with only 25 square feet of grate surface. It may be said that small grates require less manipula-

TABLE 14.

Average Rate of Combustion, lbs. per sq. ft. of grate per hour	Water F. & A. 212° per lb. dry bagasse, lbs.	Per cent CO_2 in flue gas	No. of Tests included in average
85	4.97	10.3	6
130	4.26	12.68	16
200	4.38	10.5	9

tion and care in order to prevent excessive air losses than is the case with very large grates, there being less danger of portions of the grate being uncovered, etc. Naturally, the amount of grate surface per B. H. P. will depend also on the amount of moisture in the bagasse. In other words, the less moisture there is the smaller the grate can be without danger of choking the furnace. This means that in Louisiana relatively large grates and correspondingly low rates of combustion are best.

The highest rate of combustion obtained in any of the tests was 225 pounds of bagasse per square foot of grate surface per hour at Adeline, corresponding to about 15 B. H. P. for each square foot of grate, while at Vermilion the rate of combustion was 85, corresponding to about 6.5 H. P. per square foot of grate. In both of these the boiler was operated at overload. It is probable that a mean between these two sizes would be good practice.

AIR SUPPLY.

The tests showed that with a large grate and open setting, together with a bagasse feeder that excluded air poorly, it was easy to have too much stack draft (vacuum). On the other hand, a relatively small grate in connection with a close setting and a hopper that excludes air, not only can be operated efficiently with high draft, but actually requires it. With open setting and inefficient hopper feeders, the excess air loss can be reduced by using forced (plenum) draft and the efficiency in this manner increased.

Evidently there would be less advantage in this respect for blowers where settings and feeders are close and the grates are small. For regulating stack draft it is better to manipulate flue dampers than draft doors. Closing the draft doors results in increased vacuum inside of the setting, which, in turn, results in increased air leakage, most of it above the grate, which increases the stack loss. On the other hand, closing the flue damper reduces the vacuum inside of the setting, reduces the amount of air leakage through the settling walls above the grate and, in turn, the stack loss. With the latter form of regulation the proportion of air passing through the grate is greater than with the former method. This has the advantage that the grate bars are kept cooler, due to the relatively greater amount of air passing through them. To be effective, flue dampers should be carefully designed and constructed so that they will work positively and stay where they are set. If possible, it should be arranged so that they can be manipulated by the fireman from the front of the furnace. With such an arrangement it is more likely that the fireman will actually use them. The fact of the matter is that it is not necessary for flue dampers to be changed continually. A little practice and observation will enable the firemen to determine the proper setting which is likely to be constant most of the time. The use of flue dampers also makes it possible to work all of the boilers whether far from or near the stack at equal loads.

FLUE GAS ANALYSIS.

It will be noted from the flue gas analyses that the loss due to incomplete combustion of carbon (C) was in most cases small as compared with the loss due to excess air, the latter being indicated by the amount of (O). Theoretically, that flue gas with the maximum percentage of CO_2 and the minimum percentage of CO would represent conditions most desirable. In practice, however, it is found that if the damper is set so as to get a very high percentage of CO_2 by reducing the air, there is danger of incomplete combustion and the formation of CO, due to lack of air in portions of the fuel bed. Table 15 shows typical flue gas analyses from coal furnaces.

TABLE 15.

	Poor		Average	Excellent
CO_2	8.0	9.0	12.0	15.1
O	4.4	11.5	7.5	4.0
CO	7.6	Trace	0.1	0.7

The two poor analyses are of different kinds. It will be noted that the percentage of CO_2 differs little in the two poor analyses, though there is a wide difference in the percentage of oxygen and CO. In other words, the first analysis is poor because of the large amount of CO, while the second one is poor because of the large amount of oxygen, which denotes a large percent of excess air. From the tests we are led to the opinion that 15.1 per CO_2 in gases from bagasse furnaces is rather high, in fact, 12 to 13 per cent seems to be about as high as can be obtained without lowering the efficiency. This is probably due to the cause stated above; namely, that when the air supplied is reduced to the point that will give greater CO_2 content than this, portions of the fuel bed will fail to get the necessary amount of air and there will be a corresponding loss due to incomplete combustion.

EFFICIENCY AS AFFECTED BY OVERLOAD.

The boiler H. P. bagasse will generate per ton of cane ground per 24 hours depends upon several factors, principal of which are percent fibre in cane and moisture in bagasse. In the tests of 1909 (Series A) the average B. H. P. actually developed per ton of cane ground per 24 hours was about 1 H. P. These tests were made with bagasse of relatively high moisture from cane with a low fibre content, probably a little less than 10 percent. In the 1914-15 tests the H. P. actually developed per ton of cane ground per 24 hours varied from a minimum of 1.16 at Angola, where the fibre in cane (9.8%) and efficiency was low, to a maximum of 1.44 at Vermilion, where both fibre (11%) and efficiency were high.

In practically all cases, the tests showed that boilers operated at overload gave efficiencies equal to or greater than when operated at rated or underload. It is probable that operating boilers at overload is more desirable from the standpoint of efficiency than with coal or oil burners, due to the fact that the larger furnaces cause greater radiation and air leakage losses, and these losses are a smaller percent of the total with large than with small loads. This means that for the highest efficiency flue temperatures are likely to be higher with bagasse as fuel than with coal or oil as fuel.

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Vegetable Decolorizing Carbons and Their Use in the Cane Sugar Industry

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PREFACE

This station has, during the past few months, received a great many requests for information on the preparation and uses of the active decolorizing carbons which have lately created such a stir in the sugar world. In order to satisfy this demand, it was decided to assemble in a short, non-technical bulletin all the important data existing on the subject, including the results of some laboratory and sugar house tests made at this station during the past grinding season. In publishing this information, we are well aware of the fact that our knowledge of these carbons and of their practical application is still very limited, and we hope that the present contribution, incomplete though it be, may help to clear up some questions about which there has been a difference of opinion.

The writer gratefully acknowledges the kind and efficient cooperation of Mr. W. G. Taggart and Mr. A. J. Keller, especially in the sugar house tests. Thanks are also due to Mr. A. G. Davis, who assisted in the analytical and other laboratory work.

Vegetable Decolorizing Carbons and Their Use in the Cane Sugar Industry.

By F. W. Zerban, Ph. D.

BRIEF HISTORICAL SKETCH.

Attempts at the utilization of the absorptive qualities of wood charcoal in the sugar industry date back about one hundred and twenty-five years. In 1785 Lowitz called attention to this useful property of charcoal, which had been long known before him and had been forgotten again. He subsequently made some tests on sugar syrups, but apparently without success. However, in 1794 an English refinery actually used charcoal for decolorizing raw sugars, with good results. Not many years later this same material was to play an important part in the history of the sugar industry, helping to place the young and struggling beet sugar industry on a firmer basis. It was with the aid of charcoal, in addition to other improvements, that Delessert made a practical success of the manufacture of sugar from beets at his Passy refinery, in 1808. This favorable outcome prompted Napoleon Bonaparte to cause the erection of additional factories, and thus led to the establishment of the beet sugar industry in France.[1]

But at just about that time—in 1812, the decolorizing effect of bone-black was discovered, and it was soon found that this form of carbon was superior in decolorizing power to ordinary wood charcoal. It quickly took the place of the latter, and for a number of decades following was in many places believed to be an absolute necessity for making good sugar, in both refineries and raw sugar factories. It is now no longer considered of such paramount importance, but is still being largely used by refiners.

Notwithstanding the great success of bone-black, wood charcoal was not entirely forgotten, and its good qualities as a filtering material were rediscovered several times during the past hundred years. In 1836 Boettcher recommended the use of brown coal or lignite, which is a natural carbonization product of vegetable matter, for refining sugar to be sent to the East Indian market, on account of the religious scruples of the Hindoos regarding sugar made with the aid of bone-black.[2] Around the middle of the century sugar charcoal, and, once again, brown coal were given favorable mention.[3]

About thirty years later the subject of vegetable carbons was revived anew and created a good deal of discussion at the time. There was a spirited controversy between Casamajor, chief chemist of Havemeyer & Elder, who was in favor of using finely ground crude sawdust as a filtering medium, and Remmers, of England, who, at first, recommended powdered wood charcoal, patented by himself, and afterwards a certain kind of specially pulverized lignite, patented by Kleeman.[4] Rather exhaustive tests of the last two processes were carried out in Demerara in 1886, and in Louisiana, at Magnolia, in 1886, and at Calumet in 1887; some experiments were also made about that time at the Louisiana Sugar Experiment Station.[5] Mr. W. J. Thompson, who reported on the Calumet experiments, expressed himself in favor of wood charcoal which gave a larger increase in purity, although the lignite exhibited a declorizing power about ten times as great. It is interesting to note that Remmers, in his British patent granted in 1881, describes the revivification of wood charcoal by boiling with alkali, a method which had been in use for several decades in regenerating bone-black.

After another thirty years' intermission the subject was once more taken up, as it had meanwhile been found that vegetable chars of high decolorizing power can be prepared by special methods. This fact in itself was not new, since almost one hundred years ago Bussy and other French chemists had devised methods for greatly increasing the decolorizing effect of chars of both animal and vegetable origin.[6] A series of patents for the manufacture of active vegetable carbons have been taken out since the early eighties, and their number has greatly increased during the past seven years.

Many vegetable decolorizing carbons are now on the market in this and other countries and are being used in various industries. They are sold under different trade names, like Eponit, Filtchar, Flaming, Flandrac, Littoral, Norit, etc.[27] It is difficult to ascertain how many of these have been tried in sugar factories, but as far as the writer is aware, the practical use of only a few of them has been discussed in the more widely read sugar journals. These are Eponit; Norit; the bagasse char advocated by Weinrich in 1891[7] and in a purified form by Clacher in 1914[8];

the widely discussed rice hull carbon, recently discovered in Louisiana[9]; and the char prepared from filterpress mud, according to the processes recommended by Karlik and Stanek in 1903[10], by Hazewinkel[11] in 1911, and lately by Sandmann.[12]

PREPARATION OF VEGETABLE DECOLORIZING CARBONS.

It has already been mentioned that almost one hundred years ago some French chemists found a way for increasing the decolorizing effect of chars, including those of vegetable origin. It consisted in mixing the raw material before carbonization with other substances, like chalk, flint, pumice stone, etc. These additions serve the same purpose as does the calcium phosphate of bones in the preparation of bone-black—that is, as a skeleton on which the carbon particles are deposited. A finer distribution of the carbon and a more porous structure are thus obtained. This is of importance, because the decolorizing power of carbons is evidently closely associated with their porosity, although other factors also seem to play a part. As it is not the purpose of this bulletin to discuss questions of solely scientific character, the readers specially interested in the theoretical side of this subject are referred to an article by Schneller, published in the Louisiana Planter for September 8, 1917, page 154.

It is now known that the increase in decolorizing power may be effected in several ways. It can be accomplished by simply heating the vegetable char, without any admixture, to high temperatures in the absence of air; or by first mixing the raw material or char with other "impregnating" substances and heating the mixture under conditions which prevent the oxidation of the resulting carbon. The impregnating substance is afterwards partly or wholly removed by treatment with proper solvents. Heating in a current of water vapor, or of certain gases or mixtures of gases, is also used for imparting high decolorizing power to vegetable chars.

Systematic investigations on several of the methods just mentioned have been in progress for some time at the Louisiana State University, and since last year also at the Louisiana Sugar Experiment Station. Coates, at the university, has found that ordinary wood charcoal from sawdust, the decolorizing power of which is only very slight, can be converted into a carbon equal to Norit by prolonged heating to 1200° C. in a covered crucible. This corroborates the results obtained by Lotz with lignite, in 1911.[13]

At the experiment station tests on the preparation of carbons from sawdust by means of impregnating substances were begun last year by Schneller, and they are being continued. It was found, among other things, that a carbon of the same appearance and of properties similar to Norit and Eponit, the manufacture of which is kept secret, may be prepared by mixing sawdust with milk of lime, driving off most of the water, heating the mixture in a closed receptacle in a muffle or Fletcher furnace, treating the mass after cooling with hydrochloric acid, washing and drying. When two parts of lime are used to three parts of sawdust, a carbon of the same decolorizing power as Norit is obtained, while that made from equal parts of the ingredients results superior to Norit. These results were confirmed by investigations carried out independently in England.[14]

Other active carbons were made at the experiment station by using certain metallic chlorides as impregnating substances. In 1913 Zelniczek patented the use of zinc chloride for this purpose, and that of calcium and magnesium chloride was also proposed.[15] We have found that other chlorides will give carbons which are as good or better than those prepared with the chlorides just mentioned. Tin crystals (protochloride of tin) serve as well as zinc chloride, and anhydrous magnesium chloride produces a carbon fifteen times as active as Norit, while that made with ordinary magnesium chloride is a little inferior to Norit. We have also established certain relationships between the physical properties of the impregnating chlorides and the decolorizing effect of the carbons prepared with them. These investigations will be published in detail in a technical bulletin.

Vegetable carbons of high decolorizing power have further been obtained from raw materials which are already impregnated, either by nature, or incidentally by some manufacturing process.

It was shown at the experiment station that active carbons may be prepared from those plants or parts of plants which have a high percentage of infusible ash, consisting, for instance, principally of silica, or of lime salts.

The highly silicious parts of certain grasses, like rice hulls or leaves, barley awns, etc., yield very active carbons. Cane bagasse also has ash rich in silica. The decolorizing effect of the chars made by simple carbonization of these materials is only slight, and must first be developed by removing the silica from the char. This can be effected by boiling with caustic soda solution, preferably under pressure, or by fusing with carbonate of soda or caustic soda. In the case of rice hull char, boiling with a water solution containing 20% of caustic soda on the weight of the char, has been found to give good results. After removing the soda by washing with water and a little acid, a carbon is obtained which exhibits about one and one-half times the decolorizing effect of Norit. This carbon now contains only 9% of ash, as against 50% in the char, and, according to Schneller,[16] it is useless to remove more ash by employing higher concentrations of soda, because the decolorizing power of the carbon is not further increased thereby. The quantity of soda to be used in treating the other silicious materials mentioned above will depend on the percentage of silica contained in them. The silica may also be removed by treatment with hydrofluoric acid, but it is doubtful whether this method could successfully compete with the caustic soda treatment, since the latter furnishes silicate of soda as a by-product which ought to command a good market.

The impregnating substance of the carbon from filterpress mud, advocated by Sandmann, consists largely of phosphate of lime, because the inventor proposes to use lime and phosphoric acid for clarification. The mud is dried, charred, and the decolorizing power of the char is then developed by boiling it with just enough hydrochloric acid to cause a distinctly acid reaction. About one quart of the commercial acid is required to every five to seven pounds of char. The carbon is then washed and may be dried if desired.

PROPERTIES OF VEGETABLE DECOLORIZING CARBONS AND METHODS OF EMPLOYING THEM.

The vegetable decolorizing carbons are usually sold in the form of a fine, intensely black powder. They are light in weight, their apparent density depending largely on their degree of fineness, and to some extent on the ash found in them. Their composition varies somewhat, but they are generally characterized by a high carbon and low ash percentage; they differ in this respect from ordinary bone-black, which usually has only about 10% of carbon and a very high ash content. Eponit, when perfectly dry, contains over 90% of carbon, and less than 4% of ash, and Norit has a similar composition. Eponit, according to Strohmer, has small quantities of oxygen, hydrogen, and nitrogen[17]; Coates, and also Sandmann, have found hydrogen in other vegetable carbons. Norit, however, is claimed by Wijnberg to be absolutely free of these constituents and to consist entirely of amorphous carbon with a small quantity of ash.[18]

The property which makes the vegetable decolorizing carbons especially useful is their power to remove coloring matter and certain other substances from solutions. When used on crude sugar products, they show their effect in three different ways, which are, however, to some extent interdependent. They produce a permanent and very pronounced decolorizing effect, increase the purity, and largely improve the filtrability of sugar products. Only quite small quantities of carbon are usually necessary to produce a considerable effect. The carbon is simply mixed with the solution, which is then heated for a short time and filtered. The effect is usually greater in dilute solutions than in concentrated ones. The carbons also act more efficiently when the medium is slightly acid to litmus than when it is neutral or even alkaline.

The decolorizing power of Eponit and Norit is from ten to over thirty times as great as that of ordinary bone-black, depending on the composition of the product used and the condition of the experiment. Rice hull carbon and Sandmann's carbon from filterpress mud, when properly prepared, are equal or superior to Norit. We have no figures on bagasse carbon, but its decolorizing effect is probably rather low, because dry bagasse contains only about 5% of ash and consequently still less silica.

Weinrich has lately stated that the decolorizing power of chars is determined by their volume rather than by their weight, and that equal volumes of various carbons have the same decolorizing power.[19] The results obtained at the experiment station do not bear out this contention.

The removal of other organic and mineral impurities, besides color, has lately also been questioned by Weinrich, on the basis of experiments made with the vegetable chars known about thirty years ago.[19] Weinrich claims that vegetable carbons do not remove organic and mineral impurities, but only color, while bone-black acts on all of them. This criticism has very recently been refuted by Sauer.[20] and we have also found that under the conditions used by us, Norit and rice hull carbon do remove nitrogenous substances, gums, and ash constituents, even though in small quantities. W. J. Thompson, whose work was mentioned on page 6, likewise found an increase in purity of 1.5 to 4.3 points from the use of ordinary wood charcoal on cane juice.

Some of the substances taken up by the carbon are not as firmly held as others, and are therefore more easily removed by washing. Sugar itself does not seem to be absorbed at all, and can therefore be readily recovered. This whole subject will be discussed more fully on pages 27-31.

The question as to what particular constituent of the carbons is the real decolorizing and purifying agent, has been extensively studied, as has also the other question, whether this removal of impurities is a physical or chemical process, or both, and exactly how it is brought about. Some of these problems are being studied at the university and at the experiment station, but we shall not enter into this phase of the discussion here, especially as this subject has been very ably treated by Schneller in the article already referred to.

All the advantages which we have mentioned so far would not be sufficient reason for making practical sugar house tests with these carbons, unless they were exceedingly cheap or unless they could be used repeatedly. The carbon, by taking up impurities, naturally becomes less effective after each operation. Since the carbons at present on the market are anything but cheap, the question of their repeated use becomes very important. It has been found that when relatively large quantities of carbon are employed in proportion to the impurities to be removed, the carbon can be used several times, if it is simply washed with water after each operation. But when a certain amount of foreign matter has accumulated in the carbon and its efficiency is decreased thereby, it becomes necessary to remove the impurities by some special treatment. This can be accomplished by several methods which have all been used for a long time in the revivification of bone-black. The simplest, but least effective, way consists in boiling with dilute hydrochloric acid and washing with water, which process removes some of the foreign substances, especially mineral matter. The carbon may now be used again a few times, but after this a more thorough method must be employed. For this purpose alkaline substances, like ammonia, sodium carbonate, and particularly caustic soda have been recommended. About 5% to 10% of caustic soda, calculated on the weight of the carbon, are added to the latter; the whole is mixed with water to form a rather thick paste, which is then boiled for half an hour or longer. The soda takes out a large portion of the impurities, and the solution assumes a dark brown color. The carbon is now filtered through a press, washed first with hot water, then with a little very dilute hydrochloric acid to remove the last traces of soda, and finally again with water. This method is quite effective on carbons that have not been put to very hard use, but it is rather expensive. The caustic soda method, although about a century old, has recently been patented again by Wijnberg for use with Norit and similar decolorizing materials of high carbon content.[18] The most thorough way of revivifying decolorizing carbons consists in reburning them in the absence of air, which converts all organic impurities into carbon. Then it is only necessary to remove the mineral impurities with hydrochloric acid, and to wash with water. We have found that this method completely restores the decolorizing power of carbon which was saturated with foreign matter. The inventor of Norit has patented a special kiln for the reburning of vegetable carbons.[21]

In factory practice, there is, of course, always a small loss of carbon, mostly mechanical, the extent of which will depend on the care with which the various operations are carried out. This loss has to be replaced with fresh carbon.

The revivification of carbon, which is such an important item in factory practice, is entirely avoided by the process recently proposed by Sandmann. When using any of the carbons which have to be revivified for reasons of economy, they must be added to the clarified juice and not to the raw juice. Otherwise the carbon would be contaminated with such quantities of precipitate that its revivification would become most difficult. Sandmann's carbon, however, is added directly to the raw juice, which is then, without previous filtration, acidified slightly with phosphoric acid, and at once again nearly neutralized with milk of lime. According to the inventor, a much better clarification is obtained by this method than by using the different ingredients separately and filtering after each operation. This not only saves one, or even two, filtrations, but also entirely prevents the contamination of the filtered liquor with fine carbon particles and produces a filtrate of extreme brilliancy. The mud obtained in the clarification process is again ignited and treated with muriatic acid, exactly as in the beginning. Thus a new batch of carbon is produced in every operation. The solution obtained in treating the char with muriatic acid can be neutralized with lime which will precipitate calcium phosphate. This can either be used as a fertilizer or it can be reconverted into phosphoric acid which again enters the process. In the latter case only lime, muriatic acid, and a small quantity of phosphoric acid to replace unavoidable losses would have to be bought. Every factory would be able to make its own carbon, and produce even more than is needed.

This process would, of course, render the application of manufactured carbons absolutely useless. It remains to be seen, however, whether the burning of the necessary quantity of mud for preparing the required carbon can be done economically.

PREVIOUS FACTORY TESTS.

Several ways have been suggested for the utilization of vegetable decolorizing carbons in the cane sugar industry. They might be used on juices, factory syrups, light colored raws without previous affining, and on washed tropical sugars. In the last named case the washings could also be treated with carbon, and worked into high grade consumption sugar. The same thing could be done with seconds.

A perusal of the current sugar literature would indicate that the only factory tests that have so far been reported on were made with Norit and with rice hull carbon.

The first British patent for the use of Norit in the sugar industry was granted to Wijnberg and Sauer in the year 1911.[22] The original mode of working was considerably modified in later patents, especially as regards the revivification of the Norit. Many articles on the working of this process have appeared,[23] so that it will suffice here to state only the salient points as set forth by the inventors.[24] Norit may be used for refining raws, or for making plantation whites directly from the cane. The quantity of Norit used varies from 3% on washed raw sugars to 5% to 6%, calculated on the sugar, in raw cane products. The carbon is added to the clarified juice or melted sugar, as the case may be, the mixture heated for a short time, passed first through filterpresses and then through a Danek or similar gravity filter, no other kind of filter being required in the whole operation through to the finished product. Filtration is very rapid, so that only a few presses are needed. The same Norit can be used over several times, and must then be revivified by the methods described in the foregoing chapter. The entire cycle may be repeated a number of times, and the quantity of Norit used over and over again, at least one hundred times. Only the small mechanical losses have to be replaced by adding the necessary amount of fresh Norit. The inventors claim that the cost of refining raw sugar is only 36 cents per ton in a 100-ton refinery, owing to smaller cost of machinery, operation and labor, combined with better yields and saving of time.

Experimental runs with Norit[23] were made at a cane sugar factory in Natal in 1913, and on a much larger scale again during the following year, using the carbon on slightly acid juice made by the sulphitation process. Norit was also tried at a Scotch refinery in 1913, and again in 1914, when the same quantity of the carbon was used thirty times in succession. According to Wijnberg, the process was introduced three years ago at one of the largest refineries in Canada. It appears to have been tested in Hawaii and was actually used last year for refining raws in two beet sugar factories in Holland. Experiments in Java cane factories were started in the year 1914, at the Krian factory. Here the Norit was added to the clarified, neutral sulphitation juice, together with some acid phosphate, to make the reaction distinctly acid. The published results on all these trials were very favorable. It has recently been stated[20] that the Norit process is now being used in about forty sugar factories and refineries.

The Krian factory installed the complete Norit process for the 1915-16 campaign, but several difficulties were encountered at the beginning. These were, however, remedied during the grinding season, and it was expected to take up the full operation of the process in 1916-17, in order to arrive at definite conclusions. So far, the writer has not heard anything about the results obtained during that campaign. Jentink, who saw the process worked at Krian in 1915-16, expresses the following opinion:[25] "In Java this process has not yet been adapted for work on a large scale, so that it would be useless to make comparisons with other purification methods. However, the factory tests that have been made gave very good results; the sugar which was prepared by this process may be considered superior in quality to the sugars produced so far in Java." Further on he states that the price of Norit and the cost of regenerating it will be deciding factors in judging the value of the process.

It may be said that the Louisiana Sugar Experiment Station has arrived at about the same conclusions as those expressed by

Jentink. Several runs were made last year at the sugar house of the station, under the auspices of Mr. C. D. Kemper, by a representative of the Norit White Sugar Company. Both washed and unwashed Louisiana 96 test sugar were treated with 5% Norit, after acidifying with a little phosphoric acid, and all of them boiled to grain. The washings were also treated, and grained with the molasses made before. The claims of the inventors that much time can be saved in filtration and that a high grade white sugar can be made by the process were substantiated by the results. The carbon was revivified after each operation by boiling with caustic soda. No conclusions can, of course, be drawn from these tests concerning the financial side of the question.

A test similar to the ones just mentioned was made at the sugar house of the station in July last year with rice hull carbon, at the request of some planters and under the auspices of Messrs. Blardone, Fornaris and Laroussini, of New Orleans, who also furnished the char used in the experiment. Unwashed Louisiana 96 test sugar was refined with 5.5% rice hull carbon, and the results were the same as those obtained before with Norit. Nearly a ton of sugar melted to 60 Brix was passed through 120 square feet of filterpress area in 27 minutes.[26]

LABORATORY AND SUGAR HOUSE TESTS MADE AT THE STATION DURING THE GRINDING SEASON OF 1917-18.

During the past grinding season the experiment station took up a more comprehensive study of the effect of Norit and rice hull carbon on cane juice and syrup, and made several sugar house runs in which rice hull carbon was used in connection with different methods of clarification.

Some laboratory tests were first made to study the decolorizing effect of varying percentages of Norit on juices clarified by different methods. In one series the juice of Louisiana purple cane, expressed in a laboratory mill, was used, and in each clarification experiment quantities of 0.0, 0.25, 0.5, 1, 1.5, 2 and

2.5% of Norit, on the weight of the juice, were added. In the second series we used juice from the seedling L-511, and reduced the percentages of Norit to 0.5, 1, and 1.5.

A list of the tests follows:

No. 1—Norit added to raw juice, boiled, filtered.

No. 2—Juice limed to 0.5 cc acidity, Norit added, boiled, filtered.

No. 3—Juice limed to 0.5 cc acidity, boiled, filtered; Norit added, boiled, filtered.

No. 4—Juice limed to neutrality, phosphoric acid added to 1 cc acidity, then Norit; boiled, filtered.

No. 5—Juice limed to neutrality, phosphoric acid added to 1 cc acidity, boiled, filtered; Norit added, boiled, filtered.

No. 6—Juice limed to neutrality, boiled, filtered; phosphoric acid added to 1 cc acidity, then Norit, boiled, filtered.

No. 7—Juice sulphured to 5 cc acidity, limed back to 0.5 cc acidity, Norit added, boiled, filtered.

No. 8—Juice sulphured to 5 cc acidity, limed back to 1.5 cc acidity; Norit added, boiled, filtered.

No. 9—Juice sulphured to 5 cc acidity, limed back to 0.5 cc acidity, phosphoric acid added to 1 cc acidity, then Norit, boiled, filtered.

No. 10—Juice sulphured to 5 cc acidity, limed back to 0.5 cc acidity, boiled, filtered; phosphoric acid added to 1 cc acidity, then Norit, boiled, filtered. (Used only on Louisiana Purple.)

No. 11—Juice sulphured to 5 cc acidity, limed back to 0.5 cc acidity, phosphoric acid added to 1 cc acidity, boiled, filtered; Norit added, boiled, filtered.

No. 12—Juice sulphured to 5 cc acidity, limed back to neutrality, resulphured to 1 cc acidity, Norit added, boiled, filtered. (Used only on L-511.)

The list shows that the following clarifying methods were used: No clarification in test 1; lime alone in tests 2 and 3; lime and phosphoric acid in tests 4 to 6; sulphur and lime in tests 7, 8, and 12; sulphur, lime and phosphoric acid in tests 9 to 11.

In tests 3, 5, 6, 8, 10, and 11 the juices were first treated with the clarifying agents, heated, and filtered before adding the Norit; in the remaining tests—1, 2, 4, 7, 9, and 12—the Norit was added before heating and filtering, together with the other clarifying agents employed.

It would have been desirable to determine the exact color in all of the 88 juices. But it was found that the tints obtained in one clarifying process were often so different from those produced in others that the only thing we could do (without having recourse to an exact tintometer) was to arrange all of the samples in regular order, according to depth of color only, and to pay no attention to the exact shade. Observations were made by looking horizontally through the column of liquid contained in cylinders of two inches diameter. The whole series was then divided into nine groups of increasing depth of color, as follows: 0, water white; 1, almost water white; 2, barely colored; 3, very light straw color; 4, light straw color; 5, straw color; 6, light amber; 7, dark amber; 8, brown. In classes 3 to 8 red or greenish shades were observed, depending on the method of clarification.

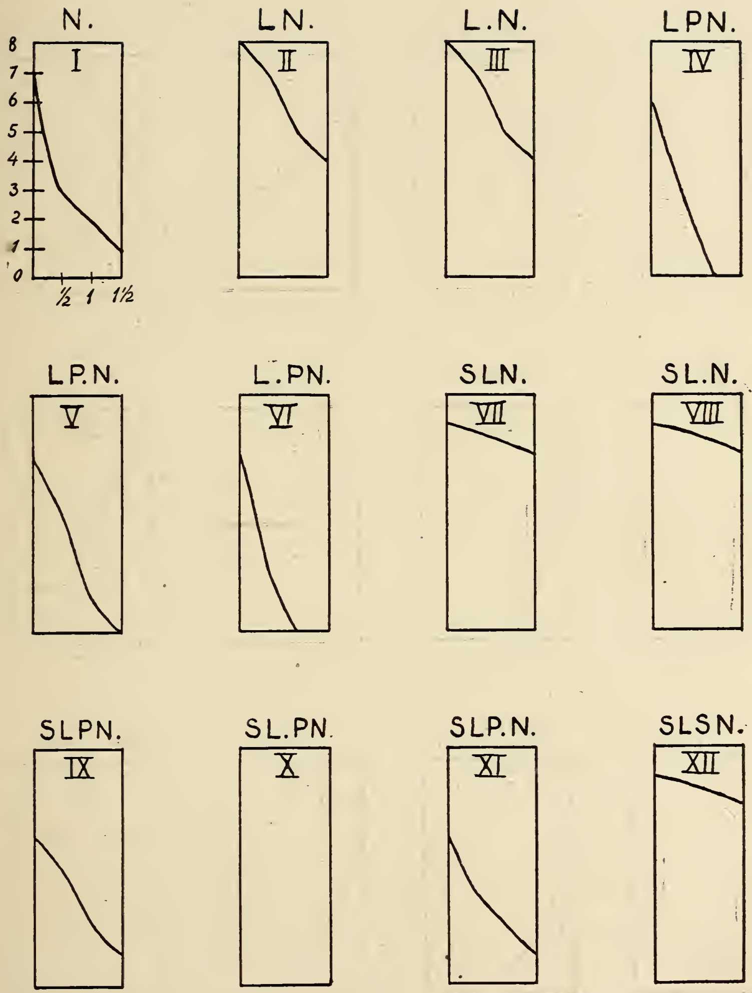
The effect of the various methods of clarification and of the Norit on the color of the juices is clearly shown in the diagrams on pages 19-20. The percentages of Norit are plotted horizontally, and the colors vertically. N signifies Norit, L lime, P phosphoric acid, S sulphurous acid, and a dot denotes boiling and filtering.

Where no Norit at all was used, the lightest colored juices were obtained by clarification with sulphur, lime, and phosphoric acid (9, 10, and 11); then follow lime and phosphoric acid (4, 5, and 6); sulphurous acid and lime (7 and 8); lime alone (2 and 3).

Where Norit was used, the highest increase in decolorization was obtained on juices clarified with lime and phosphoric acid; as little as ½% produced a strong decolorization, and with 1% the juices became practically water white. A further increase in Norit had little, if any, effect.

The carbon did not give as good results on juices clarified with sulphurous acid and lime; in the case of purple cane ½% of Norit produced only a slight effect, and it took at least 1% to reduce

L 511.


N.
I
8
7
6
5
4
3
2
1
0
½
1
1½
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II
L.N.
III
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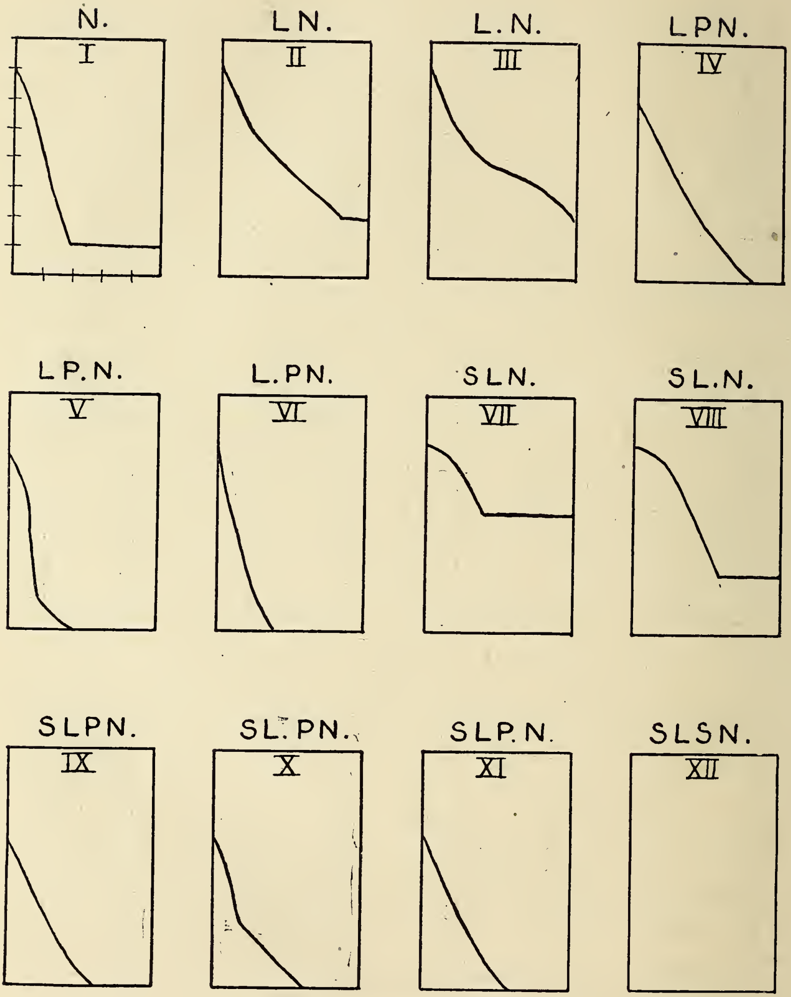
IV
L.P.N.
V
L.PN.
VI
SLN.
VII
SL.N.
VIII
SLPN.
IX
SL.PN.
X
SLP.N.
XI
SLSN.
XII

LOUISIANA PURPLE.



the color to "light straw." A comparison of juices 7 and 8 shows that a better effect is produced on the more acid juice. In the case of L-511 cane the effect of Norit was even less than with purple cane; nor did a further acidification with sulphurous acid have any effect.

In the clarification with sulphurous acid, lime, and phosphoric acid, the Norit effected a better decolorization than where only sulphurous acid and lime were used, but not as good as where only lime and phosphoric acid were employed. It took about ½% more Norit to get the same color as brought about with lime and phosphoric acid alone, although the final acidity of the juices was the same. It is impossible to explain this behavior with our present knowledge of the coloring matters of the cane.

Where only lime was used for clarification it took considerably more Norit to cause the same decolorization as obtained in the other clarification methods. This stands to reason, because the clarified juices themselves had much more coloring matter left.

The experiments teach that the least colored juices are obtained and the greatest saving of Norit can be effected by clarifying with lime and phosphoric acid. There are, however, two difficulties in the way. Phosphoric acid is a rather expensive article, as compared to sulphur, and, besides, juices clarified with lime and phosphoric acid do not settle near as well as those clarified with sulphurous acid and lime. The carbon has to take up all the mechanical impurities in the clarified juice, unless it is previously filtered, and the carbon would, therefore, have to be revivified oftener. This trouble may readily be overcome by Sandmann's process, already repeatedly mentioned. But where the carbon has to be revivified it would, perhaps, be advisable to clarify with sulphurous acid and lime, because here the clarified juice does not carry so much precipitate. A small quantity of phosphoric acid added along with the Norit to the clarified juice will cause a great improvement in color. The danger of inversion due to an acidity of 1 to 1.5 cc is only slight and is largely counterbalanced by the improvement in the color of the product. It can, moreover, be completely avoided by neutralizing the decolorized juice with lime and filtering again. The color is not affected by this after-treatment.

After the tests just described the effect of carbon on the composition of the juice was taken up in the laboratory. The different methods of clarification were again used, as above: lime alone, lime and phosphoric acid, sulphurous acid and lime, and also carbonitation. Quantities of ½% and 1% of carbon were tried. These experiments were made with rice hull carbon instead of Norit, because they were to guide us in the sugar house experiments contemplated by us, in which we had decided to use rice carbon. Where ½% carbon was added, we did not find any rise in purity in any of the juices clarified by different methods. The figures for total nitrogen and ash did not show any marked change either, nor were the gums decreased to any extent, except where lime and phosphoric acid were used in clarification. There was a fall noted in albuminoid nitrogen, but as the total nitrogen did not change materially, this might be attributed to the splitting up of albuminoids through the repeated boiling. In those tests where 1% of carbon was employed, there was an average increase in purity of about ½ point, rising to 0.9 where lime and phosphoric acid were used for clarification. We observed a uniform decrease in the total nitrogen, averaging 11.9% of the total; also a uniform and marked decrease in the gums, averaging 29.8%, and being highest in the lime-phosphoric acid clarification. The average decrease in the ash was only 5%, and in the sulphitation juice there was even an apparent slight rise. We also found again a marked decrease in the albuminoid nitrogen, as in the first set.

The analyses are not given in full, because they agreed in a general way with those obtained later on sugar house products, which are more complete.

On the basis of the data obtained in the laboratory, Mr. Taggart and the writer planned five factory experiments. This was all we could do with the small amount of cane remaining when the tests were begun. They were made with the same rice hull carbon employed in the sugar house tests last July. It had been revivified with caustic soda and was not quite as effective as fresh material.

In each of these tests a quantity of raw juice was divided into two equal parts. One half of it was run into one clarifier, and the second half into another. The two juices were in each test clarified in exactly the same way. The clarified juice from the first clarifier was then sent directly to the effects, while the other was treated with carbon, filtered through the filterpress, and then boiled to syrup, separately from the other. Each syrup was afterwards boiled to grain separately in our small pan, and purged in the centrifugal. One centrifugal charge was in each case dried without washing, while another was washed with a measured quantity of water, which was always the same for the sugar made with carbon and that made without it. In this way we hoped to obtain strictly comparable results. However, we were disappointed in this, owing to the fact that it is well-nigh impossible to boil two strikes exactly alike, especially when working with very green cane, as was the case this year, and with a small pan delivering only a hundred pounds of sugar and difficult to manage. It thus happened that in some cases we obtained a darker sugar from a lighter syrup, on account of the difficulty in purging. The molasses, however, again exhibited the lighter color corresponding to that of the syrup. It was, therefore, necessary to base our conclusions entirely on the composition and properties of the juices, syrups, and molasses. At any rate, these small scale experiments could not be expected to show more than the way by which the best results could be obtained, as far as quality is concerned, while investigations on the economic side of the problem must needs be carried out on a larger scale and over a much longer period.

The different juices, from mixed varieties, were treated as follows:

Test No. 1—Raw juice sulphured to 5.5 cc acidity, and limed back to 1.5 cc acidity. Bright juice obtained which settled well. Only scums and settlings run through the press. One half of the clarified juice brought to a boil with 1% of its weight of rice hull carbon, and passed through the filterpress.

Test No. 2—The clarification was carried out exactly like in the first experiment, with the same results, but the amount of carbon was reduced to ½%.

Test No. 3—Phosphoric acid added to the raw juice until an acidity of 3.5 cc was reached, then limed back to 1.5 cc acidity.

The clarification was poor and we found it necessary to send the entire juice through the filterpress. ½% carbon was used in this test.

Test No. 4—As we had obtained very light colored juices with the use of phosphoric acid, but at considerable expense for the acid, we decided to run another test with lime alone for clarification, and to add a small amount of phosphoric acid, ½ pound per ton of cane, at the same time when the carbon was introduced. The clarification with lime alone was poor, and all of the juice had to be filtered through the press. ½% of carbon was employed in this experiment.

Test No. 5—In this last experiment the carbon was used on the syrup instead of the juice. The raw juice was sulphured to 5.2 cc acidity, and then limed back to 0.3 cc acidity. The clarification was good, as is usually the case with carefully conducted sulphitation. All of the clarified juice was first boiled to syrup, one-half of which was then treated with two ounces of phosphoric acid per 1000 pounds of syrup, and with 5% of carbon, calculated on the weight of the sugar contained in the syrup.

All of the juices, syrups and molasses obtained in these five experiments were carefully analyzed. Measurements of the color and viscosity were made, and the speed of filtration observed, but not actually measured. We determined sucrose, reducing sugars, free acid, total nitrogen, ash, and gums. A detailed account of the reliability of the figures obtained is given under the respective headings on the following pages. The results of the analyses themselves are found in tabular form on pages 33-37. In order to make the figures comparable, the various non-sugars are, in the case of juices and syrups, expressed in per cent of total solids in the untreated products. In the molasses analyses this procedure was not possible, because unequal amounts of sugar were removed from the massecuites. We therefore based the figures in this case on equal amounts of total non-sugars (total solids less sum of sucrose and reducing sugars). Even these results are quite uncertain, on account of chemical changes taking place during the boiling, which are not under control, owing to unavoidable differences in temperature and time of boiling. For these reasons we shall in the discussion to follow consider only the averages obtained in the molasses analyses.

Color.—The clarified juice in test 1 (sulphitation with 1.5 cc final acidity) after treatment with 1% carbon showed only 17% of the coloring matter found in the untreated part. In the syrup the ratio had risen to 24%, and in the molasses to 42%. This shows that during the concentration of the liquor a large quantity of additional coloring matter is formed, which causes a rise in the proportion between the color of the treated and that of the untreated products with increasing concentration.

In test 2, where the same clarification was used as in test 1, the carbon was reduced from 1% to ½%. This reduction had a marked effect on the color of the products. The color ratio between treated and untreated products was found to be 37% in the juice, 42% in the syrup, and 51% in the molasses.

By far the lightest colored products were obtained in test 3. The juice made by clarification with phosphoric acid and lime was in itself very much lighter than that prepared by any other method of clarification. The carbon, used at the rate of only ½%, removed 70% of the small amount of color in the clarified juice, leaving only 30% in the treated part. In the syrups the ratio of the color of the treated to the untreated product rose to 35%, and in the molasses to 56%.

In test 4 only lime was used in clarification, and the color of the clarified juice was therefore rather dark. When the carbon was added, to the amount of ½%, we at the same time acidified the treated half of the juice with phosphoric acid. Since this acid itself has a pronounced decolorizing effect, the reduction in the color due to the whole treatment amounted to as much as 84%, and the color remaining was only 16%. But this advantage was quickly lost upon concentration. In the syrup the ratio was found to be 44%, and 52% in the molasses. This result may possibly be explained by the fact that in the syrups the difference in acidity was not nearly as great as in the juices.

In test 5, where the carbon was used on the syrup instead of the juice, the treated syrup showed 40% of the color of the untreated, and in the molasses the figure rose even to 60%. The ratio found in the syrups is about the same as that found where a corresponding quantity of carbon was used under similar conditions on the juice, while in the molasses it is considerably higher. The results show that the color formed during boiling is evidently not so easily removed, at least not at the natural consistenecy of syrup. These observations agree with those made by Thompson with lignite. When this material was added to the juice, it removed about one and a half to twice as much coloring matter as when used on the syrup.

Viscosity.—Contrary to our expectations, we could not find a measurable difference in the viscosity of the treated and untreated juices and syrups in any of the tests. In these investigations it was, of course, necessary to bring the untreated and treated products to the same density, as they would otherwise not have been comparable at all. But the difficulty which one encounters here, in spite of this precaution, is due to a fact established by Prinsen Geerligs in his classical researches on molasses formation. He found that the viscosity of sugar products of similar composition depends more on their absolute density than on their analysis. This in itself would explain the results obtained by us—namely, that products of the same density, but with small variations of impurities as found by analysis, showed no measurable difference in their viscosity. No viscosity determinations were made on the molasses, because here the question becomes more complicated on account of fine grain contained in them, and it would not be permissible to dissolve it with water, as was shown by Prinsen Geerligs.

It is interesting to note in this connection that both Mr. Taggart and Mr. Keller, who handled the strike pan in these tests, expressed the opinion that the treated syrups seemed to boil more freely than those which had not been treated with carbon. This would point to a decrease in the viscosity, which phenomenon, if actually present, could not be detected by the ordinary methods.

Speed of Filtration.—A great difference between the treated and the untreated products became at once apparent when we investigated the facility with which the different products could be filtered through filter paper. The treated products invariably filtered much more rapidly than the untreated ones. This is most probably due to the fact that the untreated products contain impurities in the colloidal state of aggregation; they are retained on the filter and soon clog it. In the treated products, on the other hand, these impurities are coagulated by the carbon, and the liquid therefore passes through freely. In the sugar house it was always noted that the treated products filtered much more rapidly. This was especially marked in tests 1 to 3.

It has already been mentioned that the very fine carbon particles have a tendency to run through the filter cloths at the beginning of the operation, especially in the case of sulphitation juices. This difficulty can be obviated by running the liquors back through the press again, until they filter clear. At the Krian factory in Java the same trouble was remedied by adding milk of lime to the treated juice. The precipitate formed by the lime envelops the carbon particles, and upon filtration through Danek filters an absolutely clear, bright juice is obtained, without the color being affected in the least.

Purity.—The purity of the juices was determined, as usual, by single polarization and by spindling. It was later found that during the past grinding season this "apparent" purity was very much lower than the "true" purity. The same observation was made all over the state. This explains the great difference between the purities of juices and syrups. In the latter we determined the sucrose by double polarization, and the total solids by the refractometer. In the molasses double polarization and drying were employed. While for this reason the purities of the juice, and of the syrup and molasses obtained from the same juice, are not on the same basis, those for the treated and untreated products in each stage of the manufacture, except molasses, are strictly comparable.

In test 1, where 1% carbon was used on sulphitation juice, the rise in purity in the juice amounted to 1.1 points. ½% carbon used under the same conditions (test 2) caused an increase in purity of 0.6. Where phosphoric acid was employed instead of sulphurous, and ½% of carbon, (tests 3 and 4) the increase was 0.6 and 07.

In the syrups the differences in purity were generally larger than in the juices. In test 1 the rise amounted to 1.3 points, in test 2 to 1.2, in test 3 to 1.9, in test 5 to 1.6, but in test 4 it was only 0.3 points. This last result is explained by the fact that in this experiment more sucrose was inverted, during the boiling to syrup, in the more acid, treated juice, than in the untreated. The surprisingly large increase in purity in test 3 is similarly due to a smaller difference in the glucose ratios of untreated and treated syrups than was found in the corresponding juices.

The purity of the molasses is of no value in judging the effect of the carbon, because here the amount of sugar removed by crystallization plays the most important part.

There is no doubt but that the carbon treatment causes uniformly an improvement in the purity, the extent of which depends on the method of clarification and the amount of carbon employed.

Glucose Ratio.—In test 1 the carbon treatment did not bring about a material change in the glucose ratio, either in the juice or syrup. The short boiling of the juice, when the carbon is added, evidently does not cause any inversion, even at the acidity of 1.5 cc. There was, however, an increase in the glucose ratio from juice to syrup in both treated and untreated products. The high acidity thus showed its influence upon concentration of the liquors.

In test 2 there was also no change in the glucose ratio of the juices. The syrup from the untreated juice had a slightly larger ratio than the other, evidently owing to the fact that it had been boiled to a higher density. There was again a marked increase in the ratio from juice to syrup in both treated and untreated products.

In test 3, where phosphoric acid was used instead of sulphurous, the boiling of the juice with carbon apparently caused quite a little inversion. The writer is, however, inclined to the belief that this was due to incipient fermentation rather than to the acidity, as this juice had to be kept longer than the untreated part. The difference in the glucose ratio of the syrups was not as great as in the corresponding juices. For this reason the difference in the purity of the two syrups was larger than one would have expected. Again the glucose ratio in both syrups was higher than in the corresponding juices.

In test 4, where only the carbon treated portion of the juice was acidified with phosphoric acid, there was, nevertheless, practically no inversion due to the boiling. But the higher acidity of the treated portion exerted its influence during concentration to syrup. The glucose ratio of the treated part increased 3.5 points, while that of the untreated rose only 2.1 points. For this reason the purity of the treated syrup was only 0.3 higher than that of the untreated.

In test 5, where the carbon was used on the syrup, together with a little acid, the glucose ratio of the treated syrup was actually a little lower than in the untreated.

We may conclude from these results that the carbon treatment in itself does not cause any inversion, and that any inversion which does take place upon concentration is caused by high acidity, which, however, can be avoided, as was shown in the Krian experiments, by first neutralizing the carbon treated juice with lime.

The glucose ratios in the molasses need not be considered, because they are more influenced by the acidity and by the time and temperature of the boiling than by the carbon treatment of the juice.

Free Acids.—The quantity of these is evidently not changed to any extent by the treatment with carbon. The largest change was 4.5% of the total, which is well within the limits of error. In tests 4 and 5 the acidity of the treated juices and syrups was, of course, higher than in the untreated, because in these experiments an additional quantity of acid was added along with the carbon. In the molasses the acidity was not determined, owing to the lack of reliable methods.

Total Nitrogen.—This constituent can probably be determined more accurately than any of the others. We found a small reduction of total nitrogen in all of the tests, in both juices and syrups. It averaged 8.3% of the total in juice and syrup of test 1, 8.2% in test 2, 4% in test 3, 4.9% in test 4, and 14.2% in test 5. In this particular case the carbon seemed to be more effective when used on the syrup instead of the juice.

The average total nitrogen content of the molasses from treated products was 3.9% lower than in that from untreated.

Ash.—Methods for the determination of ash are not sufficiently accurate to ascertain very slight changes, and the results are therefore not as uniform here as in the case of nitrogen. In test 1 there was a slight apparent increase, well within the limits of error. In test 2 the treated juice showed a small decrease, which was just about counterbalanced by a small increase in the syrup. In test 3, however, we found a uniform and relatively large decrease in the ash. Test 4 showed about the same relations as test 2, but in test 5 we again find a more pronounced decrease. The results on the juices are more reliable, because the total quantity of ash in the syrups is influenced by the amount of incrustation, and is therefore affected by the density to which the syrup has been boiled, as well as by other factors like acidity, time and temperature of boiling. The treated juices show a decrease in three cases out of four, and the average reduction, counting all four, is 4.8% of the total. In test 5, where the syrup was treated directly, there was also a decided reduction in the ash. The average decrease in the syrups and in the molasses was only very slight.

It would appear that the carbon causes indeed a small reduction in the ash. The actual absorption of ash by the carbon is probably somewhat larger than that found in the filtered and washed products, because mineral substances absorbed by such purifying agents as carbon are usually more readily dissolved again by washing than organic constituents.

Gums.—This term comprises a number of different constituents of cane products, about the chemical nature of which we are still quite uncertain. It usually denotes those organic, nitrogen free substances which are precipitated by strong, slightly acidified alcohol. In analytical practice the last named reagent is used for precipitation, both ash and nitrogen are determined in the precipitate, and the necessary corrections applied. The determination of gums thus depends on three analytical operations, and the accuracy of the final figure is therefore of necessity much slighter than in the case of other constituents. We have to bear all this in mind in judging the effect of the carbon treatment on the juice. In the case of the syrups the chances of error are still greater, and even more so in the molasses, because gum-like substances are readily formed during the boiling by decomposition and interaction of others, and their quantity will depend on the time and temperature of the boiling and other factors. In the syrup analyses the alcohol was acidified more strongly than in the juice tests, and the absolute figures obtained are therefore smaller. For the reasons just given we have to be guided mainly by the analyses of the juices, and to consider also that the limit of error is rather large. The actual results in the juices were as follows: 14.9% decrease in test 1, 6.3% increase in test 2, 22.1% decrease in test 3, and 39.6% decrease in test 4. In test 5, where the carbon was used on the syrup, we find an apparent increase of 5.9%. Making allowance for a rather large possible error in this last case, this would mean that any actual reduction by the carbon treatment of the syrup must have been slight. In the juices, however, we find a decrease in three cases out of four, the average of all four tests being 17.6%. It is certain that at least part of the large reduction observed in test 4 is due to the high acidity of the treated juice. But even if the reduction here had not been more than in test 3, the average decrease would still amount to 13.2% of the total. The writer feels therefore justified in concluding that the carbon really caused a reduction in the gums. This is further strengthened by the fact that in the syrups the average reduction was 13.3%, and in the molasses 19.3%.

It is probable that the difference in the various constituents would have been more pronounced, if larger quantities of carbon than ½% and 1% had been tried. Further experiments will be made on this question, but in the tests described here it was our aim to determine what happens under practical working conditions.

The fact established by these tests—that the purity of the products is improved, that certain impurities are actually removed by the carbon, and that the decolorizing effect is considerable and permanent—permits the conclusion that a larger amount of high grade sugar would be obtained by the use of decolorizing carbons than without them, or, in other words, that more molasses could be boiled back without impairing the color and quality of the product. There is no doubt but that for this reason the process merits a further investigation on a large enough scale and over a sufficiently long period to determine the economic aspect of the question. With the data we have at hand, it is impossible to decide whether it would be better to use the carbon directly on juices and syrups in the raw sugar factory, or to make raw sugar first and then refine it by means of carbon. In the latter case most of the coloring matter would go into the molasses, and the carbon would have to remove only the small quantity of color in the raw sugar. The advisability of using either of the two processes will therefore be largely determined by the difference in price of dark and light molasses which varies with different localities. In Louisiana it might pay to strive for higher quality molasses by using the carbon on juice or syrup.

SUGAR HOUSE TEST No. 1.

General Analysis.

	Treated	Untreated
Juices:		
Single Polarization	9.57	9.48
Brix	14.4	14.5
Reducing Sugars	0.97	0.95
Apparent Purity	66.5	65.4
Glucose Ratio	10.1	10.0
Syrups:		
Double Polarization	46.5	47.2
Total Solids by Refractometer	60.8	62.8
Reducing Sugars	5.88	6.06
Purity	76.5	75.2
Glucose Ratio	12.6	12.8
Molasses:		
Double Polarization	55.2	55.8
Total Solids by Drying	80.4	81.2
Reducing Sugars	15.38	15.53
Purity	68.7	68.7
Glucose Ratio	27.9	27.8

Non-Sugars.

	Treated	Untreated	Ratio, in %, Treated to Untreated
Juices:			
Free Acids, as aconitic	0.375	0.378	99.1
Total Nitrogen	0.206	0.214	96.3
Ash	3.63	3.55	102.3
Gums	1.174	1.379	85.1
Syrups:			
Free Acids, as aconitic	0.330	0.328	100.6
Total Nitrogen	0.204	0.234	87.0
Ash	3.60	3.58	100.6
Gums	0.500	0.624	80.1
Molasses:			
Total Nitrogen	1.811	2.039	88.8
Ash	35.57	39.26	90.6
Gums	3.701	5.487	67.4

SUGAR HOUSE TEST No. 2.

General Analysis.

	Treated	Untreated
Juices:		
Single Polarization	10.55	10.51
Brix	16.1	16.2
Reducing Sugars	1.03	1.03
Apparent Purity	65.5	64.9
Glucose Ratio	9.8	9.8
Syrups:		
Double Polarization	34.7	40.6
Total Solids by Refractometer	44.6	53.0
Reducing Sugars	3.70	4.54
Purity	77.8	76.6
Glucose Ratio	10.7	11.2
Molasses:		
Double Polarization	51.2	49.1
Total Solids by Drying	77.0	81.8
Reducing Sugars	14.79	18.8
Purity	66.5	60.0
Glucose Ratio	28.9	38.3

Non-Sugars.

	Treated	Untreated	Ratio, in %, Treated to Untreated
Juices:			
Free Acids, as aconitic	0.413	0.404	102.2
Total Nitrogen	0.250	0.270	92.6
Ash	3.95	4.09	96.6
Gums	1.586	1.492	106.3
Syrups:			
Free Acids, as aconitic	0.385	0.403	95.5
Total Nitrogen	0.283	0.311	90.9
Ash	4.02	3.82	105.3
Gums	0.497	0.473	105.2
Molasses:			
Total Nitrogen	2.203	2.389	92.2
Ash	33.76	37.73	89.5
Gums	3.791	4.802	78.9

SUGAR HOUSE TEST No. 3.

General Analysis.

	Treated	Untreated
Juices:		
Single Polarization	9.82	8.99
Brix	14.5	13.4
Reducing Sugars	0.95	0.76
Apparent Purity	67.7	67.1
Glucose Ratio	9.7	8.4
Syrups:		
Double Polarization	29.8	31.7
Total Solids by Refractometer	39.4	43.0
Reducing Sugars	3.12	3.12
Purity	75.6	73.7
Glucose Ratio	10.5	9.8
Molasses:		
Double Polarization	51.2	48.5
Total Solids by Drying	77.6	78.1
Reducing Sugars	15.92	18.25
Purity	66.0	62.1
Glucose Ratio	31.1	37.6

Non-Sugars.

	Treated	Untreated	Ratio, in %, Treated to Untreated
Juices:			
Free Acids, as aconitic	0.634	0.658	96.4
Total Nitrogen	0.216	0.234	92.4
Ash	3.54	4.10	86.4
Gums	0.557	0.716	77.9
Syrups:			
Free Acids, as aconitic	0.533	0.531	100.5
Total Nitrogen	0.224	0.225	99.5
Ash	3.78	4.14	91.4
Gums	0.507	0.517	98.0
Molasses:			
Total Nitrogen	2.032	2.119	95.9
Ash	35.22	38.01	92.6
Gums	4.265	3.915	109.0

SUGAR HOUSE TEST No. 4.

General Analysis.

	Treated	Untreated
Juices:		
Single Polarization	9.00	9.10
Brix	14.00	14.3
Reducing Sugars	0.95	0.95
Apparent Purity	64.3	63.6
Glucose Ratio	10.6	10.4
Syrups:		
Double Polarization	49.9	45.4
Total Solids by Refractometer	67.8	61.9
Reducing Sugars	7.04	5.71
Purity	73.6	73.3
Glucose Ratio	14.1	12.5
Molasses:		
Double Polarization	54.8	54.2
Total Solids by Drying	86.1	80.9
Reducing Sugars	18.38	16.45
Purity	63.6	67.0
Glucose Ratio	33.5	30.4

Non-Sugars.

	Treated	Untreated	Ratio, in %, Treated to Untreated
Juices:			
Free Acids, as aconitic	0.645	0.192	336.1
Total Nitrogen	0.282	0.287	98.4
Ash	3.49	3.66	95.4
Gums	0.642	1.063	60.4
Syrups:			
Free Acids, as aconitic	0.539	0.310	173.9
Total Nitrogen	0.294	0.321	91.7
Ash	4.10	3.98	103.1
Gums	0.358	0.809	44.2
Molasses:			
Total Nitrogen	2.482	2.773	89.5
Ash	37.6	36.31	104.3
Gums	5.105	6.440	79.3

SUGAR HOUSE TEST No. 5.

General Analysis.

	Treated	Untreated
Syrups:		
Double Polarization	36.3	42.1
Total Solids by Refractometer	47.0	55.7
Reducing Sugars	4.38	5.26
Purity	77.2	75.6
Glucose Ratio	12.1	12.5
Molasses:		
Double Polarization	50.4	49.8
Total Solids by Drying	82.5	84.0
Reducing Sugars	22.22	21.19
Purity	61.1	59.3
Glucose Ratio	44.1	42.5

Non-Sugars.

	Treated	Untreated	Ratio, in %, Treated to Untreated
Syrups:			
Free Acids, as aconitic	0.249	0.231	106.9
Total Nitrogen	0.208	0.243	85.8
Ash	3.42	3.73	91.8
Gums	0.293	0.277	105.9
Molasses:			
Total Nitrogen	2.373	2.079	114.1
Ash	46.03	39.48	117.6
Gums	2.772	4.029	68.8

RECAPITULATION OF RATIOS OF VARIOUS NON-SUGARS, BETWEEN TREATED AND UNTREATED PRODUCTS.

	Test 1	Test 2	Test 3	Test 4	Test 5	Average
Juices:						
Free Acids	99.1	102.2	96.4	336.1	......	99.2 (1 to 3)
Total Nitrogen	96.3	92.6	92.4	98.4	......	94.9
Ash	102.3	96.6	86.4	95.4	......	95.2
Gums	85.1	106.3	77.9	60.4	......	82.4
Syrups:						
Free Acids	100.6	95.5	100.5	173.9	106.9	98.9 (1 to 3)
Total Nitrogen	87.0	90.9	99.5	91.7	85.8	91.0
Ash	100.6	105.3	91.4	103.1	91.8	98.4
Gums	80.1	105.2	98.0	44.2	105.9	86.7
Molasses:						
Total Nitrogen	88.8	92.2	95.9	89.5	114.1	96.1
Ash	90.6	89.5	92.6	104.3	117.6	98.9
Gums	67.4	78.9	109.0	79.3	68.8	80.7

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Baton Rouge

The Deterioration of Cane Sugars in Storage; Its Causes and Suggested Measures for Its Control.

BY

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INTRODUCTION.

The investigation of sugar deterioration, of which the present publication is a complete report, has occupied a period of approximately nine years. While other reports have been published during that time, they were intended either as preliminary reports of progress, as in Bulletin 125, or as reports of collateral investigations, as in Bulletins 146 and 153. Shortly after embarking upon the main course of the investigation the writer recognized the necessity of building a broader foundation for the work by conducting several subordinate lines of research. It was mainly through these that the ideas discussed in the present publication were obtained.

In taking a retrospective view of the course over which the investigation has led, the author feels that whatever meagre success has crowned his efforts, it is, in a large measure, due to the unstinted assistance of those with whom it has been his great pleasure to be associated. The author feels constrained to admit that the success of the present investigation does not fulfill the expectations which might have been entertained for it, in view of the almost ideal conditions for research with which he has been surrounded. Whatever success has attended the author's efforts is due to the freedom which he has been allowed in following out his own ideas; to the opportunities which have been given him to take advanced training in a foreign country, and to the kind interest his associates have taken in his work and their untiring efforts in its behalf.

The writer desires to express his sincere thanks to Dr. C. A. Browne, of the N. Y. Sugar Trade Laboratory, for looking over a part of his manuscript and for many valuable suggestions given through correspondence. Also to Dr. Charles Thom and Miss Margaret Church, of the Bureau of Chemistry, for identifying the mould cultures obtained from sugars. The writer's thanks are also due to Dr. C. E. A. Winslow, of the New York Museum of Natural History, for pure cultures of the potato group of bacteria used in one of the experiments. For the kindly interest taken in his problems and for the many valuable suggestions given in connection therewith, the writer acknowledges his sincere appreciation to Prof. Alfred Koch, of the Landwirtschaftliche Institut of Goettingen, in whose laboratory two semesters were spent upon this investigation. To Mr. Inami, Mr. Hargrove and Mr. Sullivant, who respectively filled the position of assistant at various times, the author acknowledges his sincere indebtedness. Also to Assistant Director Taggart, and Dr. Max A. Schneller, research chemist, the author is indebted for very helpful suggestions and constant offers of assistance.

The investigation has been greatly facilitated at all times by the willingness of those engaged in the sugar industry in this locality to furnish samples of various materials. The writer wishes to express his thanks for such coöperation on the part of the managements of most of the sugar factories throughout the State. Also to Mr. J. M. McFetridge, superintendent of the American Sugar Refinery at Chalmette, who donated several lots of coarse granulated sugar, the writer desires to express his thanks.

Owing to the large volume of experimental data obtained in the extensive course of this investigation, it was found to be impracticable to include all of it in this report. In order to keep the volume of the report within an appropriate size, it was necessary to restrict the publication of experimental results to those considered essential to the successful demonstration of the theories which the experiments were intended to illustrate. In making this selection a careful average was taken of the results from duplicate experiments, so that the published results may be regarded as entirely representative of all of the available experimental data on the subject.

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PART I.

OBSERVATIONS UPON THE CHANGES IN THE CHEMICAL COMPOSITION OF SUGAR SAMPLES IN STORAGE.

The observation of the changes in the chemical composition of sugar samples during storage constitutes an indispensable means of obtaining the necessary preliminary data for the basis of an investigation of the causes of sugar deterioration and the influences predisposing thereto. It is by this means that we are enabled to study the relative tendency of various types of sugar either to successfully resist or to yield to the activities of those agencies which are capable under certain conditions of inducing their deterioration. It is only by such observations upon sugars in their natural condition that we can feel certain of obtaining results that are commensurate with the actions of the natural micro-flora complexes upon sugars unchanged in composition by efforts to sterilize them prior to inoculation with pure cultures. The purpose of this part of the investigation, however, was not only to observe the behavior of different types of sugar in storage, with a view of correlating as far as possible the various factors influencing it, but also to make a more extended investigation of the species occurring in sugars of various types and from various sources. It was anticipated that the last-named effort might result in the isolation from sugars of species of micro-organisms hitherto overlooked, whose demonstrated presence therein might serve to explain many apparently inconsistent results that have been previously obtained in the observation of the changes in the chemical composition of sugars in storage.

For these experiments a number of sugar samples were collected from the various plantations throughout the State during the grinding season of 1914. These samples were collected in special glass jars sent from this laboratory to the various sugar houses. The samples were thoroughly mixed promptly after their arrival at the station, then analyzed and divided into two portions, one of which was transferred to large Erlenmeyer flasks and placed in an incubator at 34°C. The other portion of the sample was kept in a tightly closed fruit jar at room temperature. There were two principal factors that from the beginning of the experiment were chosen for special study. These factors are the solid non sucrose-moisture ratio of sugars and the number of micro-organisms they contain. The solid non sucrose-moisture ratio is believed to be a reliable criterion of the keeping quality of raw sugars. It has been used as the basis for the ''factor of safety''[1] established by the Colonial Sugar Company of Australia, which states that the water in a raw sugar should not be more than half the non sucrose, or when the percentage of water in a sugar divided by 100 minus the polarization, is less than 0.333, the sugar will not deteriorate. The other predisposing factor in sugar deterioration would naturally be supposed to be the degree of infection of sugars with micro-organisms. If the causative agency in sugar deterioration is biological in nature, then the number of micro-organisms in sugar should vary directly with the rapidity of the rate of their deterioration.

[1] Cane Sugar, Noel Deerr, p. 383.

TABLE I.

Table Showing Changes in Composition of Sugar Samples in Storage.
Samples Having a Solid Nonsucrose-Moisture Ratio of (.100-.200).

Sample No.	Source and Type	ROOM TEMPERATURE				Moist.	Ratio Solids non Sucrose to Moist.	DRY BASIS			INCUBATOR DRY BASIS		Moist.	Ratio Solids non Sucrose to Moist.	No. Micro-organisms
		Date	S. P.	S. C.	R. S.			S. P.	S. C.	S. P.	S. C.	R. S.			
23	La. Sec.	1/12/15	88.53	90.25	3.83	2.18	.188	90.46	92.18	90.46	92.18	3.83	2.18	.188	120,000
"	"	2/20/15	87.74	90.59	3.34	2.20	.171	89.67	92.52	90.10	92.94	3.46	2.89	.232	
"	"	6/1/15	87.84	90.02		2.38	.195	89.94	92.12	91.79	92.89	2.31	4.03	.204	
"	"	8/7/15	87.34	89.84	3.84	2.15	.169	89.22	91.72	—	—	—	—	—	
32	La. 96 T.	1/14/15	95.89	97.38	1.37	0.72	.175	96.59	98.08	96.59	98.08	1.37	0.72	.175	8,000
"	"	2/25/15	95.95	97.24	1.56	1.10	.271	96.96	98.25	97.79	98.57	1.45	1.84	.462	
"	"	6/18/15	96.10	97.39	1.25	1.08		97.14	98.63	98.11	99.16	0.45	1.24	.398	
"	"	8/19/15	94.76	97.02	1.42	0.83	.158	96.50	98.76	—	—	—	—	—	
41	La. Y. C.	12/15/14	99.60	99.82	0.13	0.06	.150	99.66	99.88	99.66	99.88	0.13	0.06	.150	6,000
"	"	3/2/15	99.23	99.87	0.13	0.06		99.36	100.00	99.26	99.92	0.15	0.06		
"	"	6/17/15	99.60	99.90	0.10	0.10		99.70	100.00	99.46	100.00	0.13	0.16		
"	"	8/24/15	99.13	99.90	0.10	0.03		99.23	100.00	—	—	—	—	—	—

TABLE II.

Table Showing Changes in Composition of Sugar Samples in Storage. Samples Having a Solid Nonsucrose-Moisture Ratio of (.200-.300 incl.).

Sample No.	Source and Type	ROOM TEMPERATURE				Moist.	Ratio Solids non Sucrose to Moist.	DRY BASIS			INCUBATOR DRY BASIS		Moist.	Ratio Solids non Sucrose to Moist.	No. Micro-organisms
		Date	S. P.	S. C.	R. S.			S. P.	S. C.	S. P.	S. C.	R. S.			
8	Cuban 96............	9/23/14.............	96.94	97.90	1.13	0.80	.261	97.72	98.68	97.72	98.68	1.13	0.80	.261	1,300
“	“	10/23/14............	96.75	97.23	1.13	0.77	.236	97.50	97.98	98.43	98.97	0.53	1.66	.522	“
9	Cuban 96............	9/23/14.............	95.35	96.50	2.10	1.26	.270	96.56	97.71	96.56	97.71	2.10	1.26	.270	2,000
“	“	10/23/14............	95.12	95.67	2.30	0.89	.182	95.97	96.52	96.57		2.54	2.71	.453	“
10	Cuban 96............	9/23/14.............	94.80	95.96	2.52	1.33	.255	96.07	97.23	96.07	97.23	2.52	1.33	.255	4,800
“	“	10/23/14............	94.16	94.89	2.63	1.08	.184	95.18	95.91	93.80	94.27	4.45	2.67	.308	“
11	Cuban 96............	9/23/14.............	94.75	95.93	2.60	1.26	.240	95.95	97.13	95.95	97.13	2.60	1.26	.240	2,900
“	“	10/23/14............	94.54	95.73	2.86	1.04	.190	95.53	96.72	93.68	94.60	4.09	2.33	.275	“
12	Cuban 96 A.........	10/9/14.............	95.36	96.30	1.90	1.15	.247	96.46	97.40	96.46	97.40	1.90	1.15	.247	43,300
“	“	1/6/15..............	95.21	96.26	1.30	1.41	.294	96.56	97.61	94.73		2.85	4.68	.492	“
13	Cuban 96 B.........	10/9/14.............	95.28	96.08	2.05	1.06	.224	96.30	97.10	96.30	97.10	2.05	1.06	.224	26,000
“	“	1/6/15..............	95.20	95.68	2.00	1.17	.243	96.32	96.80	—	—	—	—	—	—
14	Cuban 96 C.........	10/9/14.............	95.70	96.48	1.22	0.97	.225	96.63	97.41	96.63	97.41	1.22	0.97	.225	23,300
“	“	1/6/15..............	95.41	95.73	1.10	1.38	.300	96.73	97.05	96.58	96.81	0.68	.278	.460	“
15	Cuban 96 T.........	10/9/14.............	93.67	94.63	2.77	1.59	.248	95.15	96.11	95.15	96.11	2.77	1.59	.248	66,600
“	“	1/6/15..............	93.42	94.50	2.30	1.85	.281	95.15	96.23	—	—	—	—	—	—
30	La. 96 T............	1/14/15.............	95.99	97.47	1.64	0.97	.242	96.93	98.41	96.93	98.41	1.64	0.97	.242	10,000
“	“	2/23/15.............	95.80	96.50	2.18	1.01	.244	96.77	97.47	97.70	97.80	1.72	1.95	.467	
“	“	6/5/15..............	95.91	97.78	1.73	1.01	.246	96.88	98.75	98.22	99.45	0.35	1.75	.504	
“	“	8/19/15.............	95.72	98.07	1.70	0.94	.219	96.62	98.97	—	—	—	—	—	
38	P. R. 96 T..........	1/18/15.............	95.12	95.70	2.79	1.36	.278	96.42	97.00	96.42	97.00	2.79	1.36	.278	30,000
“	“	2/27/15.............	94.71	95.45	2.86	1.19	.224	95.84	96.58	96.65	96.21	3.18	2.33	.419	
“	“	6/15/15.............	94.01	95.55	2.63	1.28	.213	95.22	96.76	94.98	94.16	3.92	3.35	.413	
“	“	8/21/15.............	94.41	95.97	2.40	1.30	.232	95.64	97.20	—	—	—	—	—	

TABLE II.---Continued.

Table Showing Changes in Composition of Sugar Samples in Storage. Samples Having a Solid Nonsucrose-Moisture Ratio of (.200-.300).

Sample No.	Source and Type	ROOM TEMPERATURE				Moist.	Ratio Solids non Sucrose to Moist.	DRY BASIS			INCUBATOR DRY BASIS		Moist.	Ratio Solids non Sucrose to Moist.	No. Micro-organisms
		Date	S. P.	S. C.	R. S.			S. P.	S. C.	S. P.	S. C.	R. S.			
28	La. 96 T.	1/14/15	96.80	97.07	1.02	0.94	.294	97.71	98.98	97.71	98.98	1.02	0.94	.294	8,000
"	"	2/20/15	97.00	97.34	1.40	0.96	.320	97.94	98.24	98.11	98.42	1.28	1.14	.380	
"	"	6/1/15	97.20	97.53	1.25	0.83	.296	98.01	98.34	98.75	98.72	0.71	2.18	.502	
"	"	8/17/15	96.90		1.03	0.71	.229	97.59	—	—	—	—	—	—	
34	La. 96 T.	1/14/15	93.78	96.04	1.13	1.89	.303	95.56	97.82	95.56	97.82	1.13	1.89	.303	8,000
"	"	2/25/15	93.13	94.68	2.60	2.09	.304	95.08	96.63	96.10	97.55	2.52	2.40	.389	
"	"	6/8/15	94.00	95.63	1.67	1.55	.258	95.48	97.11	94.28	96.24	3.97	3.38	.383	
43	La. 88°	1/18/15	86.04	87.66	5.63	4.29	.307	89.74	91.39	89.74	91.39	5.63	4.29	.307	12,000
"	"	3/4/15	85.95	87.44	5.36	4.18	.297	89.54	91.04			3.84	4.78	.441	"
"	"	6/17/15	87.97	88.78	4.36	4.42	.367	91.86	92.67	92.95	93.40	1.87	3.72	.358	"
"	"	8/24/15	87.82	89.20	4.80	3.94	.323	91.29	92.67	—	—	—	—	—	
39	P. R. (J)	1/18/15	95.11	95.23	2.54	1.55	.316	96.59	96.71	96.59	96.71	2.54	1.55	.316	44,000
"	"	3/2/15	95.02	95.74	2.16	1.71	.343	96.65	97.37	97.45	97.16	1.63	2.73	.531	"
"	"	6/15/15	94.32	96.07	2.13	1.45	.255	95.69	97.44	96.51	96.66	2.32	2.50	.427	"
"	"	8/21/15	94.81	95.96	1.63	1.50	.289	96.24	97.39	—	—	—	—	—	

TABLE III.

Table Showing Changes in Composition of Sugar Samples in Storage. Samples Having a Solid Nonsucrose-Moisture Ratio of (.300-.400 incl.)

Sample No.	Source and Type	ROOM TEMPERATURE				Moist.	Ratio Solids non Sucrose to Moist.	DRY BASIS		S. P.	INCUBATOR DRY BASIS		Moist.	Ratio Solids non Sucrose to Moist.	No. Micro-organisms
		Date	S. P.	S. C.	R. S.			S. P.	S. C.		S. C.	R. S.			
46	La. 96 T.	1/18/15	96.20	96.60	1.66	1.31	.344	97.47	97.87	97.47	97.87	1.66	1.31	.344	6,000
"	"	3/4/15	95.79	96.63	1.65	0.95	.344	96.71	97.55	96.48	97.23	1.76	1.33	.277	"
"	"	6/19/15	95.20	96.98	1.34	0.90	.187	96.06	97.84	98.47		0.08	1.29	.462	"
44	La. P. G.	1/18/15	99.50	99.42	0.06	0.17	.340	99.67	99.59	99.67	99.59	0.06	0.17	.340	7,000
"	"	3/4/15	99.40	99.79	0.08	0.03	.050	99.43	99.82	98.88	99.68	0.67	0.37	.248	"
"	"	6/19/15	99.10	99.94	0.13	0.06	.066	99.16	100.00	95.67	97.82	2.83	1.33	.237	"
"	"	8/24/15	99.00	99.54	0.08	0.06	.060	99.45	100.00	—	—	—	—	—	
37	P. R. (G)	1/18/15	95.81	96.39	1.95	1.43	.341	97.19	97.77	97.19	97.77	1.95	1.43	.341	1,460
"	"	2/27/15	95.79	96.41	2.76	0.93	.220	96.69	97.31	97.48	97.10	2.43	2.05	.457	"
"	"	6/12/15	94.99	95.28	1.64	0.96	.191	95.91	96.20	96.74	—	1.54	1.70	.347	"
"	"	8/21/15	95.41	96.94	1.75	1.12	.241	96.48	98.01	—	—	—	—	—	
45	La. 96 T.	1/18/15	95.90	96.14	1.47	1.40	.341	97.25	97.49	97.25	97.49	1.47	1.40	.341	1,000
"	"	3/4/15	95.50	96.25	1.53	1.09	.242	96.55	97.30	96.88	97.53	1.56	1.33	.302	"
"	"	6/19/15	95.30	97.15	1.40	1.03	.219	96.29	98.14	96.72	98.15	0.08	0.34	.094	"
"	"	8/24/15	96.10	97.28	0.21	1.21	.310	97.27	98.45	—	—	—	—	—	
29	La. 96 T.	1/14/15	94.63	95.97	1.65	2.00	.372	96.53	97.87	96.53	97.87	1.65	2.00	.372	633,000
"	"	2/23/15	95.33	95.23	1.64	2.00	.428	97.24	97.14	96.76	96.99	1.06	2.54	.450	"
"	"	6/5/15	94.16	—	1.43	2.74	.469	96.75	—	91.97	93.79	5.18	4.46	.372	"

TABLE IV.

Table Showing Changes in Composition of Sugar Samples in Storage.
Samples Having a Solid Nonsucrose-Moisture Ratio of (.400-.500).

Sample No.	Source and Type	ROOM TEMPERATURE				Moist.	Ratio Solids non Sucrose to Moist.	DRY BASIS			INCUBATOR DRY BASIS		Moist.	Ratio Solids non Sucrose to Moist.	No. Micro-organisms
		Date	S. P.	S. C.	R. S.			S. P.	S. C.	S. P.	S. C.	R. S.			
25	La. 2nds............	1/12/15..............	89.18	90.28	2.40	4.45	.411	93.25	94.35	93.25	94.35	2.40	4.45	.411	323,700
"	"	2/20/15..............	90.10	92.27	1.16	4.83	.487	94.46	96.63	94.13	95.42	0.37	5.45	.506	
"	"	6/1/15..............	89.78	91.23	0.98	4.45	.435	93.88	95.33	85.90	87.48	7.69	6.76	.344	
"	"	8/17/15..............	89.30	90.73	1.26	4.78	.446	93.57	95.00	—	—	—	—	—	
35	La. Y. C............	1/18/15..............	99.06	99.56	0.52	0.44	.468	99.50	100.00	99.50	100.00	0.52	0.44	.468	236,700
"	"	2/27/15..............	98.92	99.11	0.48	0.33	.305	99.40	99.59	97.76	98.40	1.87	1.19	.321	
"	"	6/12/15..............	98.89	99.43	0.77	0.26	.234	99.15	99.69	94.14	95.26	4.90	2.49	.304	
"	"	8/21/15..............	98.30	—	1.03	0.34	.200	98.64	—	—	—	—	—	—	
31	La. Y. C............	1,14/15..............	98.79	99.30	0.19	0.70	.578	99.49	100.00	99.49	100.00	0.19	0.70	.578	660,000
"	"	2/25/15..............	98.49	—	0.55	0.66	.437	99.15	100.00	98.34	—	1.45	1.06	.392	
"	"	6/5/15..............	97.29	98.68	1.26	0.66	.317	97.94	99.33	96.39	98.29	2.77	1.65	.318	
"	"	8/19/15..............	97.19	98.98	1.75	0.59	.209	97.57	99.36	—	—	—	—	—	

TABLE V.

Table Showing Changes in Composition of Sugar Samples in Storage. Samples Having a Solid Nonsucrose-Moisture Ratio of (.500).

Sample No.	Source and Type	ROOM TEMPERATURE				Moist.	Ratio Solids non Sucrose to Moist.	DRY BASIS			INCUBATOR DRY BASIS		Moist.	Ratio Solids non Sucrose to Moist.	No. Micro-organisms
		Date	S. P.	S. C.	R. S.			S. P.	S. C.	S. P.	S. C.	R. S.			
36	La. 96 T.	1/18/15	97.32	98.37	1.19	1.60	.596	98.88	99.83	98.88	99.83	1.19	1.60	.596	342,143
"	"	2/27/15	97.01	97.32	0.97	1.39	.464	98.36	98.67	97.23	97.58	1.94	2.41	.476	"
"	"	6/12/15	94.62	95.70	2.08	1.84	.342	96.37	97.45	92.66	92.40	5.78	4.39	.390	"
"	"	8/21/15	93.33	95.60	2.70	1.88	.280	95.09	97.37	—	—	—	—	—	—
26	Undried Gran.	1/12/15	97.94	97.94	0.19	2.10	1.019	100.00	100.00	100.00	100.00	0.19	2.10	1.019	741,260
"	"	2/20/15	97.62	98.32	0.037	1.72	.722	99.30	100.00	99.36	100.0	0.37	1.57	.720	"
"	"	6/1/15	99.29	99.29	0.29	0.71	1000	100.00	100.00	98.18	98.83	1.60	2.10	.546	"
"	"	8/17/15	99.09	99.79	0.51	0.21	.230	99.30	100.00	—	—	—	—	—	—

DISCUSSION OF RESULTS.

A critical examination of the preceding tables shows that a solid non sucrose-moisture ratio of 3-1 does not invariably prevent a sugar from deteriorating. Exceptions are also to be observed in the case of the non deterioration of samples in which the percentage of solids non sucrose was less than three times the percentage of moisture. But with both classes of exceptions, the inadequacy of this criterion is susceptible of explanation. On the one hand, the first noted discrepancy is most often observed in cases of white sugar, both plantation granulated and yellow clarified, and often also with sugars which have been washed up to 96 test. In such cases there is a tendency, no doubt, for the films surrounding the sugar crystals to be of a lower density than one might expect from the same ratio of solid non sucrose to moisture in a natural molasses. We find, in other words, that the validity of this factor decreases in proportion as we deviate from a film composed of a natural molasses, and begin to deal with washed sugars where the molasses films are artificially diluted. And this is what we have in washed sugar, whether it be 96 test or a white plantation sugar. In the proportion that the sugar is increased in polarization test by washing, just so much is its purity raised, and hence less solids non sucrose left in the film. Hence a lower density film is the result, and a greater tendency towards deterioration. Just the reverse is the tendency to deterioration of low grade 96 test sugar, and particularly of seconds. Here we have a film of low purity molasses, which tends to show a solid non sucrose-moisture ratio indicative of a lower density film than probably exists. In these cases it will be noted that many samples did not deteriorate, when the solid non sucrose ratio would have led one to expect a rapid destruction of sucrose in storage. In commenting upon this same question of the validity of the solid non sucrose-moisture ratio as a criterion of the keeping quality of sugars, Browne,[1] who has made a most comprehensive study of the deterioration of sugar in storage, writes as follows:

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[1] La. Planter, Vol. LIV, No. 18, 1897, p. 28.

"If we suppose no dissolved sucrose to be present the limiting ratio of $\frac{W}{100\text{-Sucrose}} = 0.25$ *is represented by a film consisting of 25% water and 75% non sucrose, and this it will be noted corresponds approximately to the percentage of water and solids in low grade molasses. The saturation of the water in low grade Philippine sugars with non sucrose ingredients would thus be the explanation of why these sugars do not deteriorate. The superior keeping quality of many molasses sugars and the susceptibility of the higher grades of soft refinery sugars to deteriorate are also thus explained."*

* * * * *"According to this viewpoint, it is wrong in principle to bring low grade sugars intended for storage, to a high degree of purity by washing, and doubly wrong when the wash water employed for this purpose is taken, as is often done, from the cooling tower or from other infected sources."* A comparison of the deterioration of the room temperature and incubator stored samples showed few cases where any acceleration of deterioration could be attributed to the higher temperature of the incubator. In most cases the greater deterioration could be directly traced to the increase in moisture absorbed in the more humid atmosphere of the incubator. We come next to an extremely interesting and significant fact in connection with the changes in the composition of sugars in storage. The significant fact to which we shall now take occasion to refer, is the successive increase and decrease in reducing sugars of the same sample at different periods of storage. It will be noted that these successive changes in reducing sugars follow a corresponding change in the moisture content, and also in the solid non sucrose-moisture ratio. Let us take, for example, Sample No. 34. Here we have a reducing sugar increase from 1.13 per cent to 2.60 per cent, while the per cent moisture was increasing from 1.89 to 2.09. However, during the next period of storage the moisture decreased to 1.59 per cent and the reducing sugar also decreased to 1.67. In the latter period there was a solid non sucrose ratio of .258 as compared with .303 during the first period. A similar change is observed in the case of Sample No. 37. The fact that sugars may decrease in reducing sugar and increase in polariza-

tion during storage has often been noted. It is most frequently observed in the case of low grade sugars having a high percentage of reducing sugar. Noel Deerr and Norris[1] called attention to this behavior of sugars in storage in their investigations of sugar deterioration. They also refer to a similar observation of Watts[2] and Tempany[3]. This phenomenon was further commented on by Owen[4] in 1916 in a paper presented before the Louisiana Planters' Association. As an illustration of this decrease in reducing sugar in a sample during storage, No. 43 may be taken as a typical case. Here we have a second sugar, with a solid non sucrose-moisture ratio of .307. The incubator sample of this sugar decreased in reducing sugar from 5.63 to 1.67 and increased in sucrose Clerget on the dry basis from 91.39 to 93.40. The writer has found that this behavior of sugar in storage seems to be definitely associated with a low factor of safety. In fact, it is the rule rather than the exception for the changes in the composition of raw sugars with a factor of less than .300 to be confined to the destruction of reducing sugar. As we have already noted, a lower factor has to be conformed to by white sugar and washed 96 test and a higher can be maintained in the case of seconds without danger of deterioration. Regarding the causes of this loss in reducing sugars during the storage of sugars, we will resume its discussion in Part II of this investigation. Suffice it here to say that this fermentation phenomenon is due to the suppression of the sucrose destroying power of certain groups of micro-organisms by the density of the film of molasses around the sugar crystals. Under this condition of supra maxima densities for the inversion of sucrose, the destruction of reducing sugar can still proceed. Hence the phenomenon can be taken as a definite indication of a density of molasses film around the sugar crystal, beyond that in which invertase can function.

As has already been stated, the sugar samples that were kept at room temperature were in jars provided with closely fitting metal covers. It must not be supposed, therefore, that the behavior of these samples in storage is entirely comparable with that of the original sugar when stored in bags or in barrels in warehouses.

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[1] The deterioration of sugar in storage. Bul. 24, Hawaiian sugar planter.

[2] Agricultural News—Vol. IV, S. 98.

[3] West Indian Bulletin, Vol. VII, No. 3.

[4] Some observations on the deterioration of sugar in storage.

Browne[1] has pointed out that deterioration frequently occurs in sugar samples when no evidence of deterioration can be detected in the stored sugar from which the sample was drawn. The reverse case is also often observed where, owing to bad warehousing, the sugars stored in bags become moist and deteriorate, while the samples being kept in stoppered bottles retain their original composition. In brief, it may be assumed that any difference in behavior of the sample and the original sugar stored in a warehouse would likely be due to the fact that the latter, being in more direct contact with the atmosphere, reflects in its behavior the varying atmospheric conditions as regards humidity. The sample would deteriorate more rapidly than the original, when the latter is exposed to a dry atmosphere, from which the sample would be protected from loss of moisture by virtue of the tightly fitting top to its container.

A significant question arises in connection with the consideration of the deterioration of sugar samples in storage. It is quite natural to assume that there must be a limit at which deterioration automatically ceases. But what is this limit, and what conditions regulate it? Thus Browne[2] finds the limit at which raw cane sugars cease deteriorating to be somewhat lower than indicated by the factor .333, and the ratio was in all cases approximately .250. Thus, to cite an example given by Browne of the limiting factor in sugar deterioration, we have the following:

TABLE IV.

	Muscovado No. 1.	Muscovado No. 2.
Polarization—August, 1910	92.70	92.65
" October, 1914	87.30	87.70
" January, 1915	87.25	87.60
Analysis—January, 1915, Water	3.04	2.88
" Sucrose Clerget	88.30	88.57
" Invert Sugar	6.25	6.31
" Ash	0.73	0.77
" Undetermined	1.68	1.47
" Ratio $\frac{\text{Water}}{\text{100-Sucrose}}$	0.260	0.252

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[1] Loc. cit

[2] Loc. cit.

The explanation of the automatic cessation of deterioration in sugar samples, which are prevented from absorbing moisture from the atmosphere, when a certain solid non sucrose ratio is reached, is in the writer's opinion to be sought for in the absence of sucrose in the molasses film, rather than in the influence of autointoxication on the part of the micro-organisms involved. In this view the writer concurs fully with Browne,[1] who, however, uses as a partial explanation of the phenomenon, the results of the experiments of Brain and Deerr[2] upon the action of deteriorative species of bacteria upon 10% sucrose solution. In the experiment referred to the authors noted that while all of the cane sugar in this inoculated solution was transformed in a very few days, the destruction of the reducing sugar does not progress beyond a certain stage, and remained constant after the tenth day. In the writer's opinion, it was not the toxic substances that inhibited the further destruction of reducing sugars by the micro-organisms, but the exhaustion of the nutrient element essential to the further development. The formula for the 10% sucrose solution employed in the experiments under consideration contains very little nitrogen, in fact, carrying only 0.1 peptone. Furthermore, for these micro-organisms sucrose is a much more readily utilizable source of energy than is either dextrose or levulose. This fact is shown in experiments of the writer[3] where in various mixtures of sucrose and dextrose and levulose singly and combined, the destruction of sucrose was always more rapid than that of the other sugars. It is to be expected, therefore, that the destruction of reducing sugar would be extremely slow in view of the exhausted state of the nutrients in the solution, after the fermentation of the sucrose had been completed.

We come next to consider the interesting observation previously cited by Browne[4] and confirmed by the writer's experiments, that, although the average 96 test sugar will not deteriorate in storage so long as its moisture non sucrose solids ratio is .333, yet once a sugar begins to deteriorate it will continue to decrease in polarization until the factor falls to approximately 0.250. To explain this fact it is necessary to regard this solid non sucrose ratio in the light of the varying density of the molasses to which it applies. The validity of this factor would seem to depend in most cases upon the fact that it connotes a certain density of the molasses film surrounding the sugar crystals. Generally speaking, the molasses films of unwashed sugar are of a density corresponding to the limit of saturation for a product of its purity. Hence a high ratio of solids non sucrose to moisture indicates a low purity molasses, and a corresponding tendency, according to the melassegenic theory of Geerligs[1] to resist crystallization at high densities. The validity of the factor of safety depends upon the fact that it connotes a density of film at which the sucrose destroying power of microorganisms is no longer possible. This applies only, of course, to natural molasses films. Now in most of the sucrose destroying types of fermentation which take place in the film, the sucrose is directly inverted, and only a small portion of the reducing sugar formed is utilized, as a result of which no material alteration of the density of the films of molasses takes place. So while the validity of the factor of safety for an undeteriorated sugar depends upon the fact that it connotes a film of such a density that inversion of sucrose cannot take place, the meaning is quite different with a deteriorated sample. In the latter case we may have a sugar in which the moisture content remains constant, but in which the ratio of non sucrose solids to moisture gradually increases by virtue of the inversion of sucrose. Obviously this gradual change from an unsafe to a safe ratio of solids non sucrose to moisture need not indicate any decreased tendency to deteriorate except that it suggests a decrease in the amount of sucrose in the film. Hence in this case the deterioration, which begins in a film of a density under the maximum for the inversion of sucrose, may result in the complete destruction of all the sucrose in the film. And this takes place in spite of the fact that the course of the fermentation proceeds through stages of solid non sucrose-moisture ratios, which would have been prohibitive of fermentation changes had they applied to the original sugar. However, it must not be inferred from this that the cessation of deterioration is always coincident with the exhaustion of the sucrose in the molasses film. On the contrary, Browne[1] has shown that the complete destruction of all of the sucrose in the molasses film of a deteriorating sugar, in a sealed sample, rarely ever occurs. The fact that deterioration ceases when sucrose still remains in the film finds a possible explanation in the fact that the invert sugar formed from the sucrose gives the solution considerably higher osmotic pressure, and hence has the same effect as an increase in the density of the film.

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[1] Loc. cit.
[2] Brain & Deerr, Bact. Flora of Hawaiian Sugar Bul. 9, Hawaiian Experiment Station.
[3] La. Bul. No. 125.
[4] Loc. cit.

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[1] Prinsen Geerligs Cane Sugar and its manufacture, p. 305.

[1] Chemical Factor in the Deterioration of Raw Cane Sugar.

TABLE VI.

Table Showing Changes in Composition of Inoculated Sugar Samples in Storage.

Sample No.	Source and Type	Date	TREATMENT	S. P.	S. C.	DRY BASIS S. P.	DRY BASIS S. C.	Moist.	R. S.	Solids Non Suc. Moist. Ratio	Micro. Per Gram.
1	P. G.	3/20/16	Cont.	99.50	99.84	99.51	99.85	0.02	—	.040	420
	"	7/26/16	"	98.80	98.98	98.88	99.06	0.20	—	.075	420
3	P. G.	3/20/16	Inoc. Bact.	99.20	99.23	99.24	99.27	0.05	—	.0625	1185
	"	7/26/16	"	98.90	99.30	99.07	99.49	0.18	0.23	.163	1185
5	P. G.	3/20/16	Inoc. Yeast	99.65	99.85	99.70	99.90	0.06	—	.171	160
	"	7/26/16	"	98.80	99.48	99.01	99.59	0.12	—	.100	160
15	Y. C.	3/20/16	Cont.	97.80	97.84	98.05	98.09	0.26	1.32	.118	8250
	"	7/26/16	"	96.80	97.57	97.18	97.96	0.40	1.80	.124	8250
16	Y. C.	3/20/16	Inoc. Bact.	97.60	98.33	98.04	98.77	0.45	1.18	.187	2225
	"	7/26/16	"	96.20	96.87	96.78	97.45	0.60	2.15	.157	2225
17	Y. C.	3/20/16	Inoc. Yeast	97.80	98.48	98.06	98.74	0.27	1.53	.122	8050
	"	7/26/16	"	96.20	97.03	96.78	97.61	0.60	2.22	.157	8050
18	La. 2nds	3/20/16	Cont.	84.00	84.57	86.33	—	2.70	5.26	.168	7650
	"	7/26/16	"	82.40	83.63	85.26	86.64	3.36	4.76	.190	7650
20	La. 2nds	3/20/16	Inoc. Bact.	84.00	85.38	86.41	87.83	2.80	5.13	.112	1467
	"	7/26/16	"	82.40	83.94	85.45	87.05	3.58	4.88	.203	1467

TABLE VII.

Table Showing Changes in Composition of Inoculated Sugar Samples in Storage.

Sample No.	Source and Type	Date	TREATMENT	S. P.	S. C.	DRY BASIS		Moist.	R. S.	Solids Non Suc. Moist. Ratio	Micro. Per Gram.
						S. P.	S. C.				
24	La. 2nds	3/20/16	Inoc. Yeast	84.20	85.64	86.42	87.92	2.60	5.13	.164	700
	“	7/26/16	“	82.80	84.25	85.36	86.80	3.00	4.76	.174	700
2	La. 96	3/20/16	Cont.	96.00	96.41	97.11	97.53	1.15	1.25	.287	1340
	“	7/26/16	“	95.50	95.73	96.77	97.01	1.32	1.06	.293	1340
4	La. 96	3/20/16	Inoc. Bact.	95.90	96.17	96.85	97.13	0.99	1.25	.241	495
	“	7/26/16	“	95.90	96.50	97.39	97.99	1.53	0.89	.373	495
6	La. 96	3/20/16	Inoc. Yeast	96.20	96.72	97.31	97.84	1.14	1.13	.300	545
	“	7/26/16	“	95.80	96.59	97.15	97.94	1.39	1.06	.330	545
7	La. 96° Washed	3/20/16	None	95.50	95.85	96.72	97.06	1.25	1.66	.277	525
	“	7/26/16	“	94.85	95.92	95.94	97.02	1.14	1.51	.221	525
19	La. 96°	3/20/16	Cont.	96.60	97.29	97.39	98.09	0.82	1.45	.241	1560
	“	7/26/16	“	95.35	96.09	96.24	96.99	0.93	1.41	.200	1560
21	La. 96°	3/20/16	Inoc. Bact.	96.20	96.45	97.11	97.36	0.94	1.48	.247	1635
	“	7/26/16	“	95.10	95.83	96.20	96.94	1.05	1.39	.214	1635
26	La. 96°	3/20/16	Inoc. Yeast	96.00	96.18	96.77	....	0.80	1.64	.200	1243
	“	7/26/16	“	94.50	95.17	95.38	96.06	0.93	1.41	.169	1243

TABLE VIII.

Table Showing Changes in Composition of Inoculated Sugar Samples in Storage.

Sample No.	Source and Type	Date	TREATMENT	S. P.	S. C.	DRY BASIS		Moist.	R. S.	Solids Non Suc. Moist. Ratio	Micro. Per Gram.
						S. P.	S. C.				
11	La. 2nds	3/20/16	None	82.20	83.36	87.91	89.15	6.50	3.17	.365	810
	"	7/26/16	"	80.00	82.37	85.45	87.98	6.38	4.35	.319	810
36	La. 2nds	3/20/16	Cont.	90.20	90.26	93.08	93.14	3.10	2.15	.316	855
	"	7/26/16	"	87.70	88.40	90.22	90.94	2.80	2.44	.232	855
37	La. 2nds	3/20/16	Inoc. Bact.	90.40	90.74	93.31	93.66	3.12	1.62	.325	18,100
	"	7/26/16	"	89.10	89.69	91.54	92.08	2.60	2.32	.238	18,100
42	La. 2nds	3/20/16	Inoc. Yeast	90.20	90.76	93.44	94.02	3.47	1.62	.354	2,157
	"	7/26/16	"	89.70	90.31	92.09	92.70	2.68	2.27	.252	2,157
29	P. G.	3/20/16	Cont.	99.30	99.91	99.37	99.89	0.08	—	.114	632
	"	7/26/16	"	98.80	99.32	98.91	99.43	0.12	—	.100	632
35	P. G.	3/20/16	Inoc. Bact.	99.40	99.65	99.44	99.69	0.05	—	.0833	670
	"	7/26/16	"	98.30	98.86	98.56	99.12	0.27	0.34	.158	670
41	P. G.	3/20/16	Inoc. Yeast	99.40	99.52	99.62	99.74	0.23	—	.383	195
	"	7/26/16	"	98.50	98.86	98.69	99.05	0.20	0.27	.133	195

TABLE IX.

Table Showing Changes in Composition of Inoculated Sugar Samples in Storage.

Sample No.	Source and Type	Date	TREATMENT	S. P.	S. C.	DRY BASIS		Moist.	R. S.	Solids Non Suc. Moist. Ratio	Micro. Per Gram.
						S. P.	S. C.				
9	96° T. Washed	1/8/16	Cont.	97.70	97.78	98.33	98.42	0.65	0.95	.239	1,135
	"	7/26/16	"	95.70	96.15	96.39	96.84	0.72	2.22	.167	1,135
10	96° T. Washed	3/20/16	Washed with 2 1.1.% Na2 HPO4	96.30	96.88	97.42	98.00	1.15	1.41	.310	1,230
	"	7/26/16	"	93.45	94.15	94.72	95.43	1.35	3.03	.206	1,230
8	96 T. Washed	3/20/16	None	96.50	97.13	97.57	98.21	1.10	1.66	.314	610
	"	7/26/16	"	93.50	94.31	94.62	95.44	1.19	3.22	.183	610
27	96 T. Washed	3/20/16	Cont.	96.80	96.81	97.69	97.70	0.92	1.04	.287	2,507
	"	7/26/16	"	94.30	94.77	95.46	95.94	1.22	1.41	.214	2,507
31	96 T. Washed	3/20/16	Inoc. Bact.	97.00	97.58	98.07	98.56	1.10	0.85	.366	16,880
	"	7/26/16	"	95.20	95.56	96.52	96.88	1.37	1.72	.275	16,880
34	96 T. Washed	3/20/16	Inoc. Yeast	96.90	97.05	98.07	98.22	1.20	0.82	.387	920
	"	7/26/16	"	94.75	95.31	96.10	96.67	1.41	2.00	.268	920
23	La. 96 T.	3/20/16	Cont.	94.00	94.27	96.06	96.34	2.15	1.71	.358	1,650
	"	7/26/16	"	91.40	92.30	93.40	94.32	2.15	2.32	.250	1,650
30	La. 96 T.	3/20/16	Inoc. Bact.	93.40	93.83	95.47	95.91	2.17	1.61	.328	709
	"	7/26/16	"	92.20	93.05	94.56	95.43	2.50	2.32	.320	709
33	La. 96 T.	3/20/16	Inoc. Yeast	93.40	93.41	95.71	95.75	2.42	1.61	.366	712
	"	7/26/16	"	91.85	92.45	93.15	93.76	2.47	2.50	.303	712

TABLE X.

Table Showing Changes in Composition of Inoculated Sugar in Storage.

Sample No.	Source and Type	Date	TREATMENT	S. P.	S. C.	DRY BASIS S. P.	DRY BASIS S. C.	Moist.	R. S.	Solids Non Suc. Moist. Ratio	Micro. Per Gram.
12	La. 96 T. Washed.........	3/20/16....	Cont......................	96.90	97.69	98.42	99.22	1.55	0.92	.500	2,200
	"	7/26/16....	"	94.35	95.03	95.72	96.41	1.44	2.44	.254	2,200
13	La. 96 T. Washed.........	3/20/16....	Washed 1 L. 5% Na_2HPO_4 .	96.80	97.53	97.77	98.51	1.00	0.80	.312	3,630
	"	7/26/16....	"	94.70	95.52	95.85	96.68	1.21	1.82	.228	3,630
14	La. 96 T. Washed.........	3/20/16....	Washed 1 L of 1% Na_2HPO_4	96.20	96.47	97.56	97.83	1.40	1.11	.368	5,407
	"	7/26/16....	"	93.50	94.27	94.84	95.62	1.42	2.50	.218	5,407
22	P. G. undried.............	3/20/16....	Cont......................	97.10	97.35	98.52	98.77	1.45	0.32	.486	987
	"	7/26/16....	"	96.10	96.61	97.27	97.79	1.21	1.48	3.10	987
25	P. G. undried.............	3/20/16....	Inoc. Bact................	97.00	97.32	98.67	99.00	1.70	0.36	.566	31,675
	"	7/26/16....	"	95.80	96.45	97.93	98.59	2.18	1.21	.517	31,675
28	P. G. undried.............	3/20/16....	Inoc. Yeast...............	97.00	97.32	98.87	99.20	1.90	0.33	.633	81,490
	"	7/26/16....	"	95.30	95.80	97.34	97.85	2.10	1.33	.447	81,490
32	P. G.....................	3/20/16....	Cont......................	99.80	99.95	99.81	99.96	0.02	—	.100	172
	"	7/26/16....	"	99.05	99.60	99.09	99.64	0.05	—	.052	172
	P. G.....................	3/20/16....	Inoc. Bact................	99.80	99.85	99.96	100.00	0.16	....	.800	1,360
	"	7/26/16....	"	98.85	99.29	99.06	99.50	0.22	0.32	.103	1,360
44	P. G.....................	3/20/16....	Inoc. Yeast...............	99.70	99.81	99.87	99.99	0.18	—	.600	202
	"	7/26/16....	"	99.10	99.24	99.18	99.32	0.09	—	.100	202
45	Y. C. undried.............	3/20/16....	None.....................	95.80	96.42	99.44	100.00	3.67	0.88	.873	4,000
	"	7/26/16....	"	94.10	94.39	96.30	96.60	2.29	2.12	.388	4,000
46	Y. C. undried.............	3/20/16....	None.....................	95.80	95.91	97.91	98.02	2.16	1.31	.514	2,007
	"	7/26/16....	"	92.80	93.37	94.59	95.17	1.90	3.12	.263	2,007

SECOND SERIES OF SAMPLES.

In the experiments with the first series of sugar samples under observation in storage, the results of which have been discussed, the plan was mainly to observe the relative susceptibilities of various types of sugar to deterioration, and to note the effects of high temperatures and relatively high humidities upon those changes.

In the experiments carried on with sugar samples the following year, an effort was made to determine, as far as possible, the influence of the degree of infection of sugar upon their deterioration. The samples were procured in much larger quantities than in the previous year, and they were thoroughly mixed upon their arrival at the laboratory, and divided into three equal portions. These triplicate samples were then treated as follows: One was inoculated with a one cc portion of a 24-hour growth of a culture of bacteria obtained from sugar. Another was inoculated from a molasses agar streak of a torula culture, and to the third was added one cc of sterile distilled water. The addition of the liquid was made in both cases with an atomizer inoculator as described by the writer in a former publication.[1] After treatment the samples were again thoroughly mixed and placed in fruit jars and kept for further observation. The first analysis of the samples was not made until a month after the inoculation, in order that an opportunity might be allowed for the effect of the treatment to be reflected in the initial analysis.

A DISCUSSION OF RESULTS.

An examination of the analytical data upon the second series of sugar samples during storage shows few cases where the inoculation may be credited with any effect upon the changes in the composition of the samples. Even in those rare cases, where the inoculation seemed to bear results in an accelerated deterioration, the moisture content of the inoculated samples was more favorable for deterioration than was that of the controls. Moreover, the quantitative bacteriological analysis of the samples, which was made a month after the inoculation, did not frequently show an increase in the degree of infection of those samples over the controls. The negative nature of the results of inoculation, as shown in these experiments, indicated that in most cases sugars contain the necessary deteriorative potential of micro-organisms, and when no deterioration takes place it is more often than otherwise due to the lack of suitable conditions for their development.

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[1] Bulletin 125, La. Experiment Station

The results of these experiments show a much larger percentage of deterioration in the samples than in the previous series. This is no doubt due to the fact that in the present series there is a much larger percentage of washed 96 test and plantation white sugar. These types of sugar are, as we have already observed, more susceptible to deterioration than their ratio of solids non sucrose to moisture would indicate. The added moisture incident to the inoculation no doubt had an accelerative action upon the deterioration of the samples, even if it did not appear to endanger their keeping qualities as judged from the factor. In the case of the second sugars with a high factor, there does not seem to be the same tendency for them in this series to lose only in reducing sugars, as was observed in the previous series of samples. We find an indication of this type of change in composition during storage only in the case of Sample No. 24. Sample No. 36, for example, deteriorated with a factor of .316, and Sample No. 11 with a factor of .365.

REGARDING THE NUMBER OF MICRO-ORGANISMS IN SUGAR, AND QUANTITATIVE METHODS FOR THEIR DETERMINATION.

We come next to a consideration of the number of micro-organisms in sugars with special reference to the adequacy of these numbers to account for the changes which sugars undergo in storage. Experimental data upon this subject indicates that the number of micro-organisms in sugars is not a reliable criterion of the tendency of sugars to deteriorate. As will be noted from the preceding tables, the bacteriological counts of sugars often show the highest degree of infection in those samples which did not deteriorate. Furthermore, these counts are below what would be expected from any ordinary substance undergoing chemical changes as the result of biological activities. It has been objected to the theory of the biological cause of sugar deterioration, that the number of micro-organisms shown in bacteriological analyses of sugars are too small to account for any detectable changes in their chemical composition. According to the work of Rahn[1] at least one million living cells of bacteria per cc. are necessary to produce any measurable changes in the composition of any substance. In sugars this number of micro-organisms per gram is rarely ever found. The writer has very rarely found sugar containing over 500,000 micro-organisms per gram. Deerr and Norris[2] report a variation in the degree of infection of sugar samples ranging from less than 100 cells per gram to 10,000. In many cases they report the number occurring in sugars with the sign of infinity, but in these cases it is likely that the excessive number refers to an overcrowding on the agar plates due to the use of too low a dilution of the sugar examined. Greig Smith[3] also reports a very small number of micro-organisms per gram in sugars that he examined, although he appears not to have made very extensive quantitative determinations.

There is one factor which has to be taken into account when interpreting the meaning of a quantitative bacteriological analysis of sugars. In dealing with sugars we are dealing with a substance which, from a bacteriological standpoint, consists of a large amount of inert and a small amount of infected material. The sugar crystal is, in all probability, a sterile substance within its interior, and the infection of its exterior is limited to the amount of molasses it carries as its film. This being the case, a quantitative bacteriological estimation of the number of micro-organisms in sugar really gives the number contained in the film of molasses surrounding the crystals. And since the ratio of molasses to sugar crystal is not always known, the number of micro-organisms as ascertained by bacteriological analysis refers to a dilution of unknown magnitude. The ratio of molasses to crystal may vary possibly from 0.1 to 10 per cent. Therefore the high counts obtained from low grade sugars may not be relatively higher for the film than the low counts obtained from high grade sugars. There is no doubt, also, that the difficulty of elaborating adequate quantitative methods has resulted in obtaining erroneously low counts in the bacteriological analyses of sugars. Owen[1] showed that the 10% sucrose agar so generally used for the quantitative bacteriological analysis of sugar gave much lower results than media of higher densities. The use of higher density media resulted in fact in showing the presence in large number of torula in sugars. The higher counts obtained from these media were almost entirely due to the fact that they admitted of the development of this group of micro-organisms, which had been almost entirely suppressed by the prejudicial osmotic effects of the low density media. The writer, however, has recently had occasion to make further observation on the subject, which shows that the principle of high density media must be carried still further to become most effective. The occasion referred to was the desire to ascertain the number of micro-organisms in fermenting cane syrup. In those experiments the counts from syrups which were undergoing vigorous fermentation appeared entirely too low, although the medium employed in the determinations was a 30° Brix molasses agar. In the quantitative bacteriological analysis of sugars the number of micro-organisms that would be expected to be present even in rapidly deteriorating samples is very indefinite, as we have noted, owing to the unknown ratio between film and crystal. But in dealing with syrups or molasses we would expect to have a development on the plates of a number of micro-organisms commensurate with the changes in chemical composition which the material is undergoing. In the case of vigorously fermenting syrups or molasses we could not feel satisfied with a count of a few thousands, or even a few hundred thousands per gram. But at the beginning of our experiments, with the use of a high density medium and dilution water, we were obtaining just such inadequate numbers in the analysis of fermenting syrup.

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[1] The fermenting capacity of the average single cell of Bacterium lactis acidi, Otto Rahn, Michigan Technical Bulletin, No. 10.

[2] Loc. cit.

[3] The Deterioration of Raw and Refined Crystals in Bulk, I. S. J., Vol. IV, 1902, pp. 430, 433, 481, 483.

After various experiments with modification of the culture medium, which consisted chiefly of varying its titre and density, it was thought advisable to substitute a syrup solution for the water in making the dilution for the plates. The results of the experiment, in which a 30° Brix syrup solution was used in comparison with water, in making the dilution for the plates, are given in the following table:

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[1] Bacteriological Investigations of Cane Sugar Products, La. Bulletin 146.

Table Showing a Comparison between the Estimation of the Number of Micro-organisms in Syrups and Sugars by the use of Water and Syrup Solution Methods of Dilution:

SAMPLE	NUMBER PER GRAM.	
	Water Dilution	Syrup Dilution
Syrup No. I	83,803	1,838,250
" No. II	106,500	1,465,000
" No. III	467,500	1,828,750
" No. IV	450,000	1,053,500
" No. V	5,100	373,350
" No. VI	15,250	2,218,500
" No. VII	297,000	1,520,000
" No. VIII	5,928	160,000
" No. IX	69,160	765,000
" No. X	....	1,187,500
" No. XI	732,500	1,184,300
" No. XII	9,550	785,230

Medium--30 Brix Syrup Agar.

The comparison between the counts obtained with the use of water and with those obtained with the syrup solution is very striking. In these experiments a careful check was made of the sterility of the syrup solution. The fact that no growth was ever obtained in plates to which the uninoculated dilution syrup was added showed that none of the higher counts obtained by this method could be accredited to the contamination of the dilution syrup. The explanation of the higher counts obtained by this method is no doubt due to the same causes which were responsible for the higher counts that were obtained with the use of high density culture media compared with media of low density.[1]

We have already offered as an explanation of the higher counts obtained in the quantitative bacteriological analysis of syrups with the substitution of syrup solution for the dilution water ordinarily used, that the former method eliminated the prejudicial effect upon the torula of the sudden change of density. If this explanation is correct, then the advantages in favor of the syrup dilution method over the water dilution method should increase in proportion to the length of the exposure of the micro-organisms to these different diluent fluids.

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[1] Loc. cit.

A test of this hypothesis was made in the following experiment. In this experiment the syrup to be analyzed was weighed into sterile measured portions of water and a 30° Brix syrup solution. Transfers from the flasks thus inoculated were made to plates immediately and after a period of two hours:

Table showing the comparative counts on syrups and sugars with the use of water and syrup solution method.

SAMPLE	WATER SOLUTION		30 BRIX SYRUP SOLUTION	
	Immediate Transfer	Transferred After 2 Hours	Immediate Transfer	Transferred After 2 Hours
Fermented Syrup	17,444	7,314	1,300,000	1,050,000
Sugar (a) Inoculated with torula	712,500	390,000	4,120,000	4,325,000
Sugar (b) inoculated with torula	737,500	63,250	2,967,0 0	1,980,000

Medium—30 Brix Syrup Agar.

The results of this experiment show that there is a great decrease in the number of micro-organisms developing on the plates inoculated from the water dilution, following an exposure of two hours. On the other hand, the result of a similar exposure to the syrup solution did not show any constant tendency either to increase or decrease. These results disprove as a possible explanation of the high counts on the syrup dilution plates, the suggestion that an increase in the number of micro-organisms takes place in the diluent solution. The use of water as a diluent fluid for the quantitative estimation of the micro-organisms in syrups or sugars obviously yields results that are misleading, since it depresses in a large measure the development of torula upon the plates. In this case we have an instance where the method of obviating the plasmolyzing effects of water as a diluent for quantitative bacteriological determinations, by the use of solutions containing a relatively large amount of solids, finds its logical application. It is interesting to note in this connection that this principle was once suggested as a modification of the water diluent method in the quantitative bacteriological determination of soils. The further investigation of this interesting observation might prove profitable from the standpoint of scientific interest. It is regretted by the writer that the lack of time prevented a more complete investigation of it. It would be particularly interesting, for example, to determine the rate of decrease in counts during certain intervals of time of exposure. Whether or not the rate of decrease would give a curve of regular or irregular slope is a question whose answer must await further investigation.

PART II.

THE MICRO-ORGANISMS CONSTITUTING THE CAUSATIVE AGENCIES IN THE CHANGES IN THE COMPOSITION OF SUGARS IN STORAGE.

A.

Bacteria.

The characteristics of the species of bacteria occurring in sugars were rather closely studied in a previous investigation of sugar deterioration, the results of which appeared in Bulletin 125. In the present investigation an effort was made to subject the various bacterial cultures isolated from sugars to a more comprehensive study, the purpose of which was to determine whether the different characteristics of the cultures were fixed, or whether they represented temporary adaptations to specific environments. The work of isolating the cultures was also conducted on a much broader basis than in the previous investigation. In the present case due significance was accorded to the idea of the possibility of the occurrence in sugars of certain species of bacteria which might not develop under the ordinary conditions of cultivation. It was therefore decided to vary the cultural conditions as broadly as possible in the attempt to isolate all species of any possible economic importance. With this in view, a series of culture media was substituted for the one medium previously used. In addition, moreover, to varying the media, the inoculated plates were exposed to both aerobic and anaerobic conditions, and to different degrees of temperature in incubation. It was thought that a combination of these various cultural conditions would result in the isolation of all of the species occurring in sugars. This work was carried on for several months, and although it resulted in a large number of cultures being obtained, yet these were reduced by successive cultivations under identical conditions to eighteen. Anaerobic and aerobic conditions, as well as low and high incubation temperatures, tend to yield quantitative rather than qualitative differences.

Having once reduced the number of cultures to what appeared to be an irreducible minimum, the work was continued further and the cultures used were grown for a number of generations upon the same medium to see if the differentiating cultural characteristics of the various cultures could be still further merged. It was found that the characteristic colony formation of the various cultures, after having been grown for a number of successive generations, under identical cultural conditions, did vary considerably from the original. However, in spite of the fact that all of the cultures did vary, yet the variation for each culture seemed to be distinctive. Hence the distinctiveness of the various cultures was as pronounced under the conditions of successive cultivations under identical conditions, as when first isolated from sugars.

Relationship to the Potato Group of Bacteria.

It was Greig Smith[1] who first pointed out the similarities of the species of bacteria occurring in sugars to the potato group of organisms. The most striking points of resemblance between the two groups are the very high resistance of their spores to heat, the ability to form gum in the fermentation of certain sugars, as well as in their general morphological and physiological characteristics. For the purpose of convenience in comparing the characteristics of the two groups, the data is condensed in the following table:

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[1] Vol. XXVI Linnear Society of New South Wales.

TABLE I.

Table Showing Characteristics of Species of Bacteria Isolated from Sugars in Comparison with Potato Species.

Culture	Size in	Flagella	Grams' Stain	Reaction to Oxygen		Liq. of Gel.	Bouillon Cult.		Spore Formation	Milk Cult.		Indol. Reaction	H_2S Product 10d	Acid from 2% Dextrose	Gas from any Sugar	Characteristics Exp. Numerically
				Aerobic	Anaerobic		Film	Cloudy		Coag.	Reaction					
Bac. vulgatus	L1-M6-5.0 B-0.8	+	+	+	+	+	+	+	+	+	Alk.	–	+	2.3	–	121.2025
Bac. mesentericus.	L-0.8-2.4 B-0.7-2.9	+	+	+	+	–	–	+	+	+	Alk.	T	++	3.00	–	122.2020
B. lioder mos	L3.5 B1.2	+	+	+	–	+			+	+					–	121.2020
B. mes. ruber	L-1.4 B-0.4	+	+	+	+	+			+	–					–	121.2020
Cult. I	L-4.7 B-1.0	+	+	+	+	+	+	+	+	+	Acid	T	++	2.4	–	121.2025
Cult. II	L-3.0 B-1.4	+	+	+	+F	+	+	+	+	+	Acid	T	–	2 4	–	121.2025
Cult. III	L-2.8 B-2.0	+	+	+	–	+S	+	+	+	+S	Acid	T	–	2.4	–	111.2020
Cult. IV	L-3.4 B-1.7	+	+	+	+	+S	+	+	+	+S	Acid	T	++	1.7	–	121.2025
Cult. V	L-3.3 B-1.4	+	+	+	+	+S	+	–	+	+S	Acid	T	++	2.3	–	121.2025
Cult. VI	L-4.9 B-1.4	+	+	+	+F	–	+	+	+	+S	Acid	T	+	1.88	–	122.2025
Cult. VII	L-3.9 B-1.1	+	+	+	+F	+S	+	+S	+	+S	Acid	T	++	2.00	–	121.2025

TABLE I---Continued.

Culture	Size in M.	Flagella	Grams' Stain	Reaction of Oxygen		Liq. of Gel.	Bouillon Cult.		Spore Formation	Milk Cult.		Indol. Reaction	H₂S Product 10d	Acid from 2% Dextrose	Gas from any Sugar	Characteristics Exp. Numerically
				Aerobic	Anaerobic		Film	Cloudy		Coag.	Reaction					
Cult. VIII...	L-3.4 B-1.2.....	+	+	+	+	+S	–	+S	+	+S	Acid	T	++	2.2	–	121.2025
Cult. IX....	L-4.0 B-1.5.....	+	+	+	+	+S	+S	+	+	+S	Acid	T	++	2.3	–	121.2025
Cult. X.....	....	+	+	+	+	+VS			+	+S	Acid		+	2.1	–	121.2025
Cult. XI....	L-3.7 B-1.2.....	+	+	+	+	+S	–	+	+	+S	Acid	T	++	2.1	–	121.2025
Cult. XII....	L-4.0 B-1.8.....	+	+	+	+		–	+	+	+S	Acid	T	++	2.3	–	121.2025
Cult. XIII...	L-3.2 B-1.4.....	+	+	+	+	+S	–	+	+	+S	Acid	T	+	1.85	–	121.2025
Cult. XIV...	L-3.4 B-1.3.....	+	+	+	+	+S	+	+	+	+S	Acid	T	++	1.7	–	121.2025
Cult. XV....	L-3.8 B-1.2.....	+	+	+	+	+S	+	+	+	+S	Acid	T	+	2.7	–	121.2020
Cult. XVI...	L-3.5 B-1.9.....	+	+	+	+	+S	+	+	+	+S	Acid	T	+	1.88	–	121.2025
Cult. XVII..	L-2.2 B-1.0.....	+	+	+	+	+VS	+	+	+	+S	Acid	T	+	1.88	–	121.2025
Cult. XVIII.	diam. 1.0	–	+	+	+F	+VS	–	+	–	+S	Acid	T	++	3.6	–	221.2020

T Trace
+ Moderate.
++ Abundant.
All acidities in milk very slight.

S—Slow.
V. S.—Very Slow.
F.—Feeble.

A critical examination of the above table shows that each of the various cultures obtained from sugars tends to conform to one or the other of the principal species of the potato group of bacteria. Just as we find liquefaction and non-liquefaction of gelatin as the respective characteristics of two of the species of the potato group, so we find the same variation in this respect in the culture obtained from sugars. In size as well as in the ability to liquefy gelatin, the majority of cultures from sugars show a closer relationship to *Bac. vulgatus* than to *Bac. mesentericus.* And this similarity also agrees to a certain extent with the relative sucrose destroying power of the different cultures. Rapid gelatin liquefaction seems usually to be associated with a strong sucrose destroying ability on the part of the cultures.

In the numerical system[1] of recording the characteristics of bacterial species, which we have used in the last column of our table, we have applied the decimal ending .0005 to gum fermentation of sucrose, and the ending .0000 to signify the lack of this ability on the part of the cultures. It will be noted that culture III, culture XV and culture XVIII lack the ability to induce the gum fermentation in sucrose solutions. These three cultures appear to take no part in the deterioration of sugars, nor do they seem to have any appreciable influence upon sucrose solutions. Their frequent occurrence in raw sugars, however, made a study of their cultural and physiological characteristics of some importance. These three species constitute the principal inert species of bacteria in sugar. Greig Smith[2] describes the inert bacteria in the Australian sugars as belonging to the streptothrix group. The term inert is used in this connection to indicate a normal rather than an abnormal state of these species. It refers to the inability of the species to cause the deterioration of sugars, even under the most favorable conditions.

Culture III and culture XV exhibit most of the characteristics of the potato group of bacteria. The former is a short, thick bacillus, the latter conforms in size to the average cells of *Bac. vulgatus.* Neither III nor XV developed any gum-forming power in the course of successive development in sucrose solutions. Culture III appears to be a strict aerobe, as no growth from it has been obtained under anaerobic conditions. Culture XVIII is a small micrococcus of 1 c.c. diameter. The frequency with which these species occur in sugars suggests a resistance on their part to plasmolyzing influence of high density solutions. The presence of a micrococcus in beet sugars was noted by A. Schone,[1] who claimed that this group constituted the largest part of the microflora of these sugars.

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[1] Chart of the American Association of Bacteriologists.
[2] Loc. cit.

The identity of the species of bacteria in sugars with the potato group of bacteria is clearly indicated in the morphological and physiological characteristics of the individual species in the two groups. The supposition seems also to admit of easy confirmation, since we have had no trouble in isolating from the soil, in the field at a distance from the sugar factory, cultures of bacteria which would conform in characteristics to the potato group, and would at the same time induce the characteristic gum fermentation of sucrose. But the conditions of those experiments did not preclude the possibility of the species thus isolated from the soil having been previously acclimatized to sugar solutions at the sugar factory and subsequently reintroduced into the soil. To obviate this possibility, pure cultures of the potato species of bacteria were obtained from the Museum of Natural History of New York City, through the courtesy of the curator, Dr. C. E. A. Winslow. These cultures were transferred to flasks of sterile 10 per cent sucrose solution to compare their sucrose destroying ability with that of a culture isolated from sugars. At the same time transfers were made from the museum cultures to tubes of 10% sucrose solution for the purpose of acclimatizing these cultures to sugar solutions. Transfers from the inoculated tubes of sucrose solutions to test tubes were made at the end of every 48 hours. After one week of this acclimatization to 10% sucrose solution these cultures were used to inoculate a second set of flasks. A second acclimatization period preceded the third experiment, so that the cultures used in this experiment had been grown for two weeks in a ten per cent sucrose solution and had been brought into contact with fresh medium every 48 hours. Coincident with this acclimatization of the cultures of the potato species to sugar solutions, a reverse process was conducted whereby an attempt was made to lower the sucrose destroying power of a species isolated from sugar, by growing it for successive generations in bouillon.

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[1] Deutsche Zuckerindustrie, 1906 Bd. 31, S. 1837.

The following table shows a comparison of the sucrose destroying power of unacclimatized cultures of the potato group of bacteria with that of a culture isolated from sugars.

TABLE II.

PART II---BACTERIA.

Table Showing Ability of Potato Species isolated from Sources other than Sugar Products, to acquire the power of destroying Sucrose.

Smith's 10% Sucrose Solution.

Cult.	Brix	Sucrose		1st Inoculation		Acidity 10 cc Rq.-cc N/10	Decrease in S. P.	Increase in R. S.
		S. P.	S. C.	Reducing Sugar	Viscosity			
Control..........	11.22	9.80	9.72	0.09	1.34	0.40	—	—
B. mesentericus...	11.52	9.50	9.49	0.10	1.33	0.84	0.30	0.01
B. liodermus......	10.98	9.50	9.40	0.15	1.33	0.80	0.30	0.06
B. mes. vulgatus...	9.98	5.40	Gum	4.17	1.77	0.60	4.40	4.08
B. mes. ruber.....	10.98	8.60	Gum	1.02	1.60	1.00	1.20	0.93
Cult X. Bouillon...	9.98	1.00	Gum	5.70	3.30	0.80	8.80	4.61
Cult X.10% SS sol.	10.36	1.30	Gum	5.70	3.27	0.80	8.50	3.18

2 Weeks Inoculation.

Second Inoculation with Cultures Grown for one Week in S. S.

Transferred Every 48 Hours.

Cult.	Brix	Sucrose		Reducing Sugar	Viscosity	Acidity 10 cc Rq.-cc N/10	Decrease in S. P.	Increase in R. S.
		S. P.	S. C.					
Control..........	10.43	9.50	9.64	—	1.26	0.30	—	—
B. liodermus......	10.49	9.50	9.64	—	1.25	0.50	—	—
B. mesentericus...	10.57	9.30	9.44	—	1.26	0.90	0.20	—
B. mes. ruber.....	10.57	3.00	Gum	6.89	2.23	0.90	6.50	6.89
B. vulgatus.......	10.33	0.40	Gum	7.54	2.77	0.60	9.10	7.54
Cult. X 10% S. S..	10.33	0.95	Gum	10.80	3.53	0.60	9.05	10.80
Cult. X. Bouillon..	10.63	1.10	Gum	11.00	3.21	0.80	8.40	11.00

TABLE III.

PART II A.---BACTERIA.

Table showing ability of potato species of bacteria isolated from sources other than sugar products to acquire the power of destroying sucrose.

(3rd inoculation after two weeks transferring.)

Cult.	Brix	Sucrose		Reducing Sugar	Viscosity	Acidity 10 cc Rq.-cc N/10	Decrease in S. P.	Increase R. S.
		S. P.	S. C.					
Control	10.43	9.40	9.56	—	1.25	0.1	—	—
B. mes.	10.61	9.35	9.21	—	1.25	0.95	0.05	—
B. liodermos	10.16	9.30	9.56	—	1.25	0.60	0.10	—
B. mes. ruber	10.19	2.90	Gum	6.55	3.70	0.70	6.50	6.55
B. vulgatus	10.19	0.80	Gum	7.54	3.02	0.80	8.60	7.54
Cult. X. S. S.	9.49	1.00	Gum	7.40	3.08	0.80	8.40	7.40
Cult X. Bouillon	10.23	0.60	Gum	7.69	3.08	0.60	8.80	7.69

Summary of data on the previous Experiments upon the acquired Sucrose Destroying Powers of the Potato Species.

Cult.	1st Experiment			2nd Experiment			3rd Experiment		
	Decrease S. P.	Increase R. S.	Increase Viscosity	Decrease S. P.	Increase R. S.	Increase Viscosity	Decrease S. P.	Increase R. S.	Increase Viscosity
B. mes.	0.30	0.01	—	0.20	—	—	0.05	—	—
B. liodermos	0.30	0.06	—	—	—	—	0.10	—	—
B. mes. vulgatus	4.40	4.08	0.43	9.10	7.54	1.51	8.60	7.54	1.77
B. mes. ruber	1.20	0.93	0.26	6.50	6.89	0.97	6.50	6.55	2.45
Cult. X Bouillon	8.80	4.61	1.96	8.40	11.00	1.95	8.80	7.69	1.83
Cult. X. S. S.	8.50	3.18	1.93	9.05	10.80	2.27	8.40	7.40	1.83

It will be noted that only B. mes. ruber and B. mes. vulgatus acquired any sucrose destroying power, and the full power was recorded after a second transfer.

A careful examination of the summarized data of the three tables shows that only *B. vulgatus* and *B. mes. ruber* acquired an increased ability to destroy sucrose in a ten per cent sucrose solution. The increase in loss of sucrose in the second experiment over the first was very striking, particularly in the case of *B. mes. ruber.* This culture destroyed more than five times as much sucrose after the first acclimatization period as it did in the first experiment. In the case of *B. vulgatus* the increase was less striking, although very pronounced. It is interesting to note that the complete sucrose destroying ability seemed to have been acquired during the first acclimatization period, as no further increase was noted in the third inoculation. Contrary to what might have been expected, the cultivation of the species of sugar bacteria in sucrose free bouillon did not tend to decrease its sucrose destroying power in ten per cent sucrose solution. From these experiments it appears evident that the cultures of potato bacteria when unacclimatized to sugar solutions do not possess as pronounced sucrose destroying ability as when grown for successive generations in contact with sucrose. This fact alone would tend to explain the great variation in gum fermenting power of cultures of sugar species isolated from different sugars. The question that arises in the light of the data shown in the preceding tables, is whether or not cultures of the potato species which have been acclimatized to ten per cent sucrose solution would be able to induce as relatively great a deterioration of higher density solutions.

TABLE IV.

Part II.

Table showing the action upon 34 Brix S. S. of Potato Bacteria Acclimatized to 10% S. S. and a Comparison with Sugar Species.

Cult.	Brix	Sucrose		Reducing Sugar	Acidity 10 cc Rq.-cc N/10	Decrease in S. P.	Increase in R. S.
		S. P.	S. C.				
Control	34.70	32.90	32.56	—	0.40	—	—
B. vulgatus	35.65	5.60	—	27.02	1.00	27.30	27.02
B. mes. ruber	34.85	6.40	—	25.00	1.20	26.50	25.00
Cult. X. Bouillon	35.15	13.00	—	22.72	0.90	19.90	22.72
Cult. X. 10% S. S.	35.15	4.00	—	27.76	0.80	28.90	27.76

Having acquired a sucrose destroying power from having been acclimatized to 10% S. S., B mes. vulgatus and B. mes. ruber were as active in destroying S. S. as the cultures obtained from sugar

The data shown in the preceding table answer this question conclusively. The acclimatized cultures of the potato species of bacteria show as striking a sucrose destroying power upon a 34 Brix sucrose solution as upon a ten per cent solution. It is also interesting to note that the culture of the sugar species, which was grown in bouillon, showed a considerably lower sucrose destroying power than the same culture cultivated in a sucrose solution.

In connection with these experiments upon the acquired ability on the part of species of bacteria to ferment sucrose solutions, it is interesting to note the observations of Kohn.[1] In his investigations upon water bacteria he found that species of those bacteria can be acclimatized to fairly high concentrations of sugar. The reversal of the process, i. e., the acclimatization to dilute solutions, take place much more rapidly than the former. He found that the limit of concentration for all of the species seemed to have been entirely governed by the osmotic pressure of the substance. Of more direct relation to the discussion of our data on acclimatization to sugar solution is the work of Landowsky,[2] who found that in plates to which transfers were made from a 30 per cent sodium chlorid bouillon, inoculated from garden soil, only *Bac. mesentericus* colonies developed. The result of that experiment, however, only indicated a resistance of the spores of *Bac. mesentericus* to high density salt solutions, for even in 25 per cent sodium chlorid bouillon that species would not develop. The resistance of the species of potato bacteria to the plasmolyzing effects of high density solution is no doubt due to the fact that they belong, as Fischer[3] has pointed out, to the group of bacteria which have a completely permeable cell membrane.

Whether or not the spores of the sugar species of bacteria are capable of developing in solutions of as high densities as those in which the vegetative cells of the species develop is a question of great practical importance as well as of scientific interest. Since most of the bacterial infection of sugars must result from the development of spores, it is of more than passing importance to determine if the maximum density in which spores can develop differs materially from the maximum for the vegetative cells. To determine whether such a variation exists between the spores and vegetative cells of the sugar species, the following experiment was conducted: A series of nutrient sucrose solutions was prepared, varying in density from 16 to 60 Brix. These solutions were then tubed and sterilized by the intermittent method of sterilization. They were then inoculated from a 24-hour growth of all the cultures of sugar species, as well as from spore preparations of the cultures. The spore preparations were made by drying smears of old agar culture upon cover glasses, at a temperature of 125° for thirty minutes. The spore preparations were several months old and in an absolutely dry condition. It was scarcely possible, therefore, that they could have contained any living vegetative cells. The spores were introduced into the sterile tubes by rubbing a sterile platinum loop over the smeared surface of the cover glass, and then gently thrusting it into the tube.

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[1] Weitere Beobachtungen Saccharophobe Bakterien cent. f. Bakt. Abt. II. XVII, 446.

[2] Ueber das Wachstum von Bakterien in Salzlosungen von Hoher Koncentration Archiv. f. Hygiene, Bd. XLIV, 1904, p. 47.

[3] Vorlesungen uber Bacterien.

TABLE V.

Experiment to Determine Limits of Density for the Development of Bacteria Cultures obtained from Sugar.

Culture	Nature of Inoculation	A		B		C		D		E		F		G		H	
		I. B.	F. B.	I. B.	F. B.	I. B.	F. B.	I. B.	F. B.	I. B.	F. B.	I. B.	F. B.	I. B.	F. B.	I. B.	F. B.
		16.6	18.20	26.15	—	30.38	31.24	30.10	36.40	40.03	40.54	42.24	43.6	44.95	46.14	55.37	55.44
		Effect Inoc.	T. R. D.	E. I.	T. R. D.	E. I.	T. R. D.	E. I.	T. R. D.	E. I.	T. R. D.	E. I.	T. R. D.	E. I.	T. R. D.	E. I.	T. R. D.
I	Veg. Cult....	+	24 hrs.	+	24 hrs.	+	24 hrs.	+	36 hrs.	+	48 hrs.	+	72 hrs.	+	96 hrs.		
	Spores......	+	48 hrs.	+	60 hrs.	+	72 hrs.	+	72 hrs.	+	72 hrs.	+	96 hrs.	+	6½ days		
II	Veg. Cult....	+	12 hrs.	+	12 hrs.	+	12 hrs.	+	48 hrs.	+	96 hrs.	+	96 hrs.	+	5½ days		
	Spores......	+	36 hrs.	+	36 hrs.	+	36 hrs.	+	84 hrs.	+	96 hrs.	+	5½ days	+	6 days		
Mixed Culture Sugar	Veg. Cult....	+	12 hrs.	+	12 hrs.	+	12 hrs.	+	48 hrs.	+	96 hrs.	+	96 hrs.	+	6 days		
IV	Veg. Cult....	+	24 hrs.	+	24 hrs.	+	60 hrs.	+	60 hrs.	+	—	+	84 hrs.	+	96 hrs.		
	Spores......	+	24 hrs.	+	60 hrs.	+	60 hrs.	+	60 hrs.	+	84 hrs.	+	84 hrs.	+	96 hrs.		
VI		16.73	16.87	26.75	26.77	30.45	30.62	35.65	35.61	40.45	40.70	42.83	42.90	45.12	45.31	55.22	55.44
	Veg. Cult....	+	24 hrs.	+	24 hrs.	+	48 hrs.	+	72 hrs.	+	72 hrs.	+	84 hrs.	+	6 days		
	Spores......	+	36 hrs.	+	48 hrs.	+	48 hrs.	+	96 hrs.	+	96 hrs.	+	96 hrs.	+	6 days		
VII	Veg. Cult....	+	24 hrs.	+	24 hrs.	+	36 hrs.	+	48 hrs.	+	72 hrs.	+	84 hrs.	+	5 days		
	Spores......	+	48 hrs.	+	36 hrs.	+	48 hrs.	+	48 hrs.	+	96 hrs.	+	96 hrs.	+	6 days		
VIII	Veg. Cult....	+	24 hrs.	+	24 hrs.	+	24 hrs.	+	48 hrs.	+	72 hrs.	+	84 hrs.	+	5 days		
	Spores......	+	48 hrs.	+	48 hrs.	+	48 hrs.	+	96 hrs.	—	—	—	—	—	—	—	—
IX	Veg. Cult....	+	24 hrs.	+	24 hrs.	+	24 hrs.	+	48 hrs.	+	72 hrs.	+	84 hrs.	+	5 days		
	Spores......	+	48 hrs.	+	48 hrs.	+	48 hrs.	+	72 hrs.	+	96 hrs.	—	—	—	—		

I. B.—Initial Brix.
F. B.—Final Brix.
E. I.—Effect of Inoculation.

T. R. D.—Time required to develop—hours.
+—Growth.
— —No. growth.

TABLE V.---Continued.

Experiments to Determine the Limits of Density for the Development of Bacteria Isolated from Sugars.

Culture	Nature of Inoculant	B		C		D		E		F		G	
		Initial Brix	Final Brix	I. B.	F. B.	I. B.	F. B.	I. B.	F. B.	I. B.	F. B.	I. B.	F. B.
		26.99	26.91	30.33	31.45	35.77	36.73	40.63	42.47	43.40	44.06	45.65	46.88
		E. I.	T. R. D.	E. I.	T. R. D.	E. I.	T. R. D.	E. I.	T. R. D.	E. I.	T. R. D.	E. I.	T. R. D.
XI	Veg. Cult.	+	12 hrs.	+	60 hrs.	+	60 hrs.	+	84 hrs.	+	5 days		
	Spores	+	72 hrs.	+	72 hrs.	+	72 hrs.	+	84 hrs.				
XII	Veg. Cult.	+	24 hrs.	+	24 hrs.	+	36 hrs.	+	60 hrs.	+	60 hrs.	+	72 hrs.
	Spores	+	36 hrs.	+	36 hrs.	+	48 hrs.	+	60 hrs.	+	84 hrs.		
XIII	Veg. Cult.	+	24 hrs.	+	24 hrs.	+	36 hrs.	+	60 hrs.	+	72 hrs.	+	72 hrs.
	Spores	+	36 hrs.	+	48 hrs.	+	84 hrs.	+	84 hrs.				
XIV	Veg. Cult.	+	12 hrs.	+	24 hrs.	+	36 hrs.	+	60 hrs.	+	72 hrs.	+	72 hrs.
	Spores	+	24 hrs.	+	36 hrs.	+	48 hrs.	+	60 hrs.	+		+	
XVI	Veg. Cult.	+	24 hrs.	+	36 hrs.	+	36 hrs.	+	48 hrs.	+	60 hrs.	+	6 days
	Spores	+	36 hrs.	+	60 hrs.	+	72 hrs.	+	96 hrs.	+	6 days	+	7 days
XVII	Veg. Cult.	+	24 hrs.	+	36 hrs.	+	36 hrs.	+	60 hrs.	+	60 hrs.	+	6 days
	Spores	+	36 hrs.										

I. B.—Initial Brix.
F. B.—Final Brix.
E. I.—Effect of Inoculation.

T. R. D.—Time required to develop.
+—Growth
——No Growth.

A study of the preceding table shows that the time required for any development to be observed in the tubes inoculated from the spore preparations was usually longer than in the tubes inoculated from the 24-hour cultures. In the lower series the difference in the time required for the development of spores and vegetative cells tends to remain constant throughout several of the various solutions. At the other extreme of the series the difference decreases, which is probably explained by the fact that in Series G the density was nearing the limit for the development of the vegetative cells as well as for the spores. The variation in the time of development of spores as well as vegetative cells does not increase directly with the density of the solution. There also appears to be a decided variation between the different cultures. An interesting fact in connection with this experiment is the application of the results to the principle of intermittent sterilization. It is obvious that the three successive day methods of sterilization could not be effectively used in the sterilization of high density sugar solution, if the solution carried as a part of its infection the spores of the sugar species of bacteria. Hence the three-day method of sterilization is still less applicable to the sterilization of sugars, that is, if it be *designed* to *destroy these* spores.

From the data in the tables under discussion it appears that the limit of density for all of the species of bacteria in sugars lies between 46 and 55 Brix. This is lower than in other experiments, where in certain instances growth was observed in solutions of slightly under 60 Brix sucrose solution. In the present experiments, however, some inversion had taken place in the sucrose solution, due to the successive heating of the tubes. It is quite likely, therefore, that the osmotic power of the solution had been sufficiently raised to depress the maximum density from the limit at which growth could have taken place in a pure sucrose nutrient solution.

TOLERANCE OF ACID AND ALKALI BY THE SPECIES OF SUGAR BACTERIA.

Since sugars vary considerably in acidity it is of importance to know the effect of the reaction of the medium upon the growth of these bacteria. For this purpose an experiment was carried on upon a 10% sucrose solution, to which varying amounts of acid and alkali were added. The media were titrated before inoculation and again at the end of the experiment.

TABLE VI.

Toleracen of Acid by Cultures of Bacteria.

7/31/15

Culture	ACID – H_2SO_4											
	I		II		III		IV		V		VI	
	10 cc N/1-100 Titration		6 cc N/1-100 Titration		4-100 Titration		2.5-100 Titration		1-100 Titration		0.4-100 Titration	
	Before Steriliza-tion	After End of 3 Days	Before Steriliza-tion	At End of Exp...	Before Steriliza-tion	At End of Exp.	Before Steriliza-tion	At End of Exp.	Before Steriliza-tion	At End of Exp.	Before Steriliza-tion	At End of Exp.
Culture	8.8	5.6	5.6	5.6	3.7	4.0	2.1	2.0	1.2	1.3	0.6	0.6
I	–		–		–		–		–		+	
II	–		–		–		–		–		+	
III	–		–		–		–		–		+	
IV	–		–		–		–		–		+	
V	–		–		–		–		–		+	
VI	–		–		–		–		–		+	
VII	–		–		–		–		–		+	
VIII	–		–		–		–		–		+	
IX	–		–		–		–		–		+	
X	–		–		–		–		–		+	
XI	–		–		–		–		–		+	
XII	–		–		–		–		–		+	
XIII	–		–		–		–		–		+	
XIV	–		–		–		–		–		+	
XV	–		–		–		–		+		+	
XVI	–		–		–		–		–		+	
XVII	–		–		–		–		–		+	
XVIII	–		–		–		–		–		+	
B. vulgatus	–		–		–		–		–		+	
B mesentericus	–		–		–		–		–		+	

TABLE VII.

Tolerance of Acid by Cultures of Bacteria.

Culture	I		II		III		IV		V		VI	
			ACETIC ACID									
	Titration		Titration		Titration		Titration		Titration		Titration	
	Before Steriliza-tion	After Steriliza-tion	Before Steriliza-tion	After Steriliza-tion	Before Steriliza-tion	After Steriliza-tion	Before Steriliza-tion	After Steriliza-tion	Before Steriliza-tion	After Steriliza-tion	Before Steriliza-tion	After Steriliza-tion
Culture	8.8 cc	8.8 cc	5.6	5.6	3.9	3.4	2.0	1.8	1.3	1.3	0.8	0.8
I	–		–		–		–		–		+	
II	–		–		–		–		–		+	
III	–		–		–		–		–		+	
IV	–		–		–		–		–		+	
V	–		–		–		–		–		+	
VI	–		–		–		–		–		+	
VII	–		–		–		–		–		+	
VIII	–		–		–		–		–		+	
IX	–		–		–		–		–		+	
X	–		–		–		–		–		+	
XI	–		–		–		–		–		+	
XII	–		–		–		–		–		+	
XIII	–		–		–		–		–		+	
XIV	–		–		–		–		+		+	
XV	–		–		–		–		–		+	
XVI	–		–		–		–		–		+	
XVII	–		–		–		–		–		+	
XVIII	–		–		–		–		–		+	
B. vulgate	–		–		–		–		–		+	
B. mes.	–		–		–		–		–		+	

TABLE VIII.

Tolerance of Alkalinity by Cultures of Bacteria. **7/3/15.**

Culture	ALKALIES. NaOH.									
	I		II		III		IV		V	
	10-100 Titration		6-100 Titration		4-100 Titration		2.5-100 Titration		1-100 Titration	
	Before Sterilizatn	After Sterilizatn	Before	After	Before	After	Before	After	Before	After
Culture	–	5.8	4.6	2.40	3.2	1.1	1.5	0.6	0.7	0.3
I	–		–		–		–		+	
II	–		–		–		–		+	
III	–		–		–		+		+	
IV	–		–		–		–		+	
V	–		–		–		–		+	
VI	–		–		–		–		+	
VII	–		–		–		–		+	
VIII	–		–		–		–		+	
IX	–		–		–		–		+	
X	–		–		–		–		+	
XI	–		–		–		–		+	
XII	–		–		–		–		+	
XIII	–		–		–		–		+	
XIV	–		–		–		+		+	
XV	–		–		–		–		+	
XVI	–		–		–		–		+	
XVII	–		–		–		–		+	
XVIII	–		–		–		–		+	
B. vulgate	–		–		–		–		+	
B. mes.	–		–		–		–		+	

Note low tolerance of cultures towards alkalinity.

The following tables give the data from the experiment. The degree of acidity or alkalinity is expressed in terms of cc of N/10 Na(OH) or H_2SO_4 required to neutralize 10 cc of the solution. It will be noted that the limit of acidity for the majority of cultures was about 0.6 to .8 cc. There did not appear to be any appreciable difference in the degree of tolerance to acetic and to sulphuric acid. The tolerance of the cultures to both acid and alkali seems remarkably low. Lehmann and Newmann[3] report an excellent development of both *Bac. vulgatus* and *Bac. mesentericus* in media, showing a reaction of+2 and —1, Fuller's scale. Edson and Carpenter[4] report excellent growth of the micro-organisms isolated from maple sap in media having an optimum reaction of +10. It was to be expected that the tolerance of acids and alkalies in a sugar solution would be different from the result obtained in bouillon, but such a low tolerance to these substances was not anticipated.

THE RELATIVE SUCROSE DESTROYING POWER OF THE VARIOUS CULTURES OF BACTERIA OBTAINED FROM SUGARS.

The fact that the various cultures of bacteria obtained from sugars vary greatly in their ability to destroy sucrose has been pointed out by all who have investigated the subject of sugar deterioration. Lewton Brain and Noel Deerr[1] determined this variation on 10% sucrose solution. Greig Smith[2] also referred to this variation in his descriptions of the bacterial species in sugars. In order to determine this variation in the sucrose destroying power of the cultures of bacteria under investigation, the author decided to test it upon sucrose solutions of higher density than those previously used. With this in view, a nutrient sucrose solution was made of about 52 Brix, using the nutrient salts in the proportions prescribed in Smith's formula for 10% sucrose solution.

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[1] Lehmann and Newmann Medical Atlas.
[2] Micro-organisms of Maple Sap, Bul. 167, Vermont Eqp. Station.
[3] Loc. cit.
[4] Loc. cit.

The following table shows the results of a 30-day incubation period of the inoculated flasks:

TABLE IX.

Experiment on the Comparative Deteriorative Powers of Different Cultures of Bacteria upon 50% S. S.

Inoculation	Sucrose		Reducing Sugar	% Total Solids	Period of Incubation	Decrease Sucrose	Increase R. S.
	S. P.	S. C.					
Control	51.50	52.44	0.03	52.81	30 days	—	—
Cult. I	26.40	—	18.80	53.76	“	25.10	18.77
“ II	25.40	—	19.20	53.76	“	26.10	19.17
“ III	51.90	52.40	0.01	53.33	“	0.04	—
“ IV	26.80	—	19.05	53.80	“	24.70	19.02
“ V	15.20	—	25.60	53.55	“	36.30	25.57
“ VI	12.00	—	29.40	53.37	“	39.50	29.47
“ VII	12.00	—	30.90	53.22	“	39.50	30.87
“ VIII	14.20	—	27.80	53.22	“	37.30	27.77
“ IX	17.00	—	26.90	52.97	“	34.50	26.87
“ X	15.20	—	27.50	53.30	“	36.30	27.47
“ XI	9.60	—	30.20	53.53	“	41.90	30.17
“ XII	27.80	—	17.50	52.80	“	23.70	17.47
“ XIII	10.60	—	29.40	53.05	“	40.90	29.37
“ XIV	11.80	—	27.80	53.30	“	39.70	27.77
“ XV	52.26	52.73	0.80	53.61	“	—	0.77
“ XVI	51.22	51.95	0.91	53.13	“	0.28	0.88
“ XVII	52.00	52.40	0.02	53.13	“	0.04	—
“ XVIII	52.52	53.02	0.02	53.77	“	—	—
Vulgatus	42.64	—	7.90	53.29	“	8.86	7.87

NOTE: Strong deteriorative power of Cult. XI.
Low deteriorative power of Cult. XVI which has high gum-forming power on 10% S. S.
Non gum-forming species no action on 50% S. S.

It will be noted that there is a great variation in the sucrose destroying power of the various cultures. This variation covers a range of 41 in the case of Culture XI to 24 in the case of Culture IV. Some of the cultures, which had been found to cause a rapid deterioration of 10% sucrose solution, were inactive in the above experiment. An unacclimatized culture of *Bac. vulgatus* was used in the experiment, which accounts for the comparatively small destruction of sucrose. The inert cultures of bacteria, III, XV, XVIII, were used in conjunction with the other cultures, and, as was to be expected, they induced no change in the composition of the solution. We have already referred to the fact that much of this variation in the sucrose destroying power of bacterial cultures isolated from sugars may be due to the respective stages of acclimatization which the different cultures may have reached. In the above experiment, however, all of the cultures, with the exception of *Bac. vulgatus*, which was grown in plain agar, had been to an equal degree acclimatized to sugar solution.

The difference in the sucrose destroying power of the various cultures of bacteria occurring in sugars has been offered as an explanation of the different behavior in storage of sugars of the same type. It has not been our experience that sugars vary to any great extent in the complexity of their bacterial infection. The difference in the bacterial infection of various types of sugar seems to be a difference of degree and not of kind. A Cuban or Porto Rican 96-test sugar is, so far as we have been able to observe, likely to carry the same species of bacteria as are found in sugars from Hawaii or Louisiana. The bacterial infection of all sugars originates from the same source, *i. e.,* from the potato group of bacteria. It is very likely true that the original bacterial infection of the majority of sugars is composed of both acclimatized and unacclimatized cultures. The former class comes from the dust and old sugar products in the factory. The latter class is composed of the spores of the bacteria in the raw juice, which persist throughout the milling process.

THE ACTION OF PURE CULTURES OF SUGAR BACTERIA UPON STERILIZED SUGARS.

In the previous experiments upon the limits of density within which the cultures of sugar bacteria can destroy sucrose, we found that it was comparatively low in all cases. No growth, in fact, was obtained in any case in the 55 Brix solution. This limitation of density seems to preclude the possibility of these cultures causing the deterioration of sugars, except in rare instances where the molasses film is greatly diluted by the absorption of moisture. The following table gives the results of a one-month incubation period of sugars inoculated from pure cultures of sugar bacteria. The sugar was sterilized for one hour at 212°, then allowed to absorb moisture under a bell jar prior to inoculation.

TABLE X.

PART II A.

Experiment on Inoculation of Sugars.
Sugar from Sugar House.
Sterlized at 212°

(Inoculation 1 month). **(Inoculation from plain Agar Streak).**

Culture	% Moist.	R. S.	Sucrose		Dry Basis		No. Micro. per Gram.	Factor
			S. P.	S. C.	S. P.	S. C.		
....	3.11	0.84	94.6	95.75	97.63	98.82	5,333	.575
II.......	3.00	0.87	94.6	95.75	97.52	98.71	32,000	.555
III......	3.11	0.83	94.6	95.75	97.63	98.82	120,900	.575
IV.......	2.91	0.80	94.8	96.12	97.64	99.00	89,300	.559
V........	3.00	0.84	95.2	96.26	98.14	99.23	1,800	.624
VI.......	3.18	0.86	95.4	96.58	98.53	99.75	39,030	.691
VII......	2.88	0.82	94.10	95.36	96.88	98.19	71,500	.488
VIII.....	3.00	0.81	94.40	95.60	97.32	98.55	130,500	.535
IX.......	2.91	0.86	95.30	96.24	98.15	99.12	28,200	.619
X........	3.18	0.87	95.00	96.18	98.12	99.34	2,376	.636
XI.......	2.61	0.83	95.40	96.48	97.95	99.06	35,000	.566
XII......	2.89	0.83	95.90	96.88	98.75	99.76	10,300	.704
XIII.....	2.91	0.86	95.00	95.83	97.84	98.71	5,500	.582
XIV......	2.96	0.90	95.10	96.07	98.00	99.00	64,000	.604
XV......	2.64	0.82	95.50	96.21	98.09	98.82	1,700	.586
Control...	4.06	0.86	94.00	94.72	97.97	98.73	....	.676

In the above table it will be noted that although the ratio of solids non sucrose to moisture was very low, giving a factor, which in some cases was as high as .704, yet no appreciable deterioration took place.

Table XI shows the results of another experiment upon the inoculation of sterilized sugar with Cultures I and II sugar bacteria, and also with Culture I torula, the latter also isolated from sugar. The sugar used in this experiment was of the washed 96-test type. It was sterilized at 212° for one hour upon intermittent days, allowing for two-three days' interval between the sterilization periods. After sterilization, the sugar was allowed to absorb moisture and inoculated as in the previous experiment.

TABLE XI.

Inoculation Experiment. Sugar from Station Sugar House. Sterilized and Inoculated in 1 Liter Flasks. 7/31/15.

Inoculation	Sucrose		Reducing Sugar	Moist.	Period of Incubation	Dry Basis		Decrease in Sucrose	Increase in R. S.
	S. P.	S. C.				S. P.	S C.		
Original Sample...	97.20	98.88	0.80	0.90	30 days	98.08	99.77	—	—
Control...........	88.80	90.32	1.49	7.89	"	96.40	98.06	—	—
Cult. I Yeast.....	91.70	93.10	0.32	5.95	"	97.50	98.99	+0.93	−1.17
Cult. I Bact.......	78.90	82.31	10.00	6.37	"	84.27	87.91	−10.15	+8.51
Cult. II Bact......	91.50	93.04	1.18	5.47	"	96.79	98.41	+0.35	−0.31

Sterilization in autoclave at 212° F. for one hour on 3 days at 2-3 day intervals. Inoculated with platinum loop of a 24-hour agar streak culture.

NOTE: Rapid deterioration of sample inoculated with Bact. Cult. and decrease in reducing sugar in sample inoculated with yeast.

The results of this experiment show a pronounced ability on the part of Culture I bacteria to destroy sucrose. The flask inoculated with Culture II bacteria did not deteriorate, which is probably explained by the fact that it contained nearly one per cent less moisture. We might well inquire why the culture of bacteria caused a deterioration of the sugar in this case and none in the previous experiment. We find the ratio of solids non sucrose to moisture approximately the same in both cases. The explanation of this difference is to be found in the difference in the types of sugars used in the two instances. In the second experiment we used a washed 96-test sugar, a type which our experience has shown to be more susceptible to deterioration than is unwashed sugar of the same grade. This fact was noted in Part I of this publication. The results of these experiments show the characteristic glucose destroying power of the torula, which was also discussed in Part I. A further discussion of it will be reserved for the next chapter.

The effect of the inoculation of sugars with bacteria will be treated more fully in a subsequent chapter of this publication. It is nevertheless opportune to discuss here in connection with the characteristics of these species the role played by them in sugar deterioration. *A critical review of the data now extant on this subject shows that no positive results have been obtained from the inoculation of sterile sugars with pure cultures of bacteria, except in cases where the sugar used would be considered abnormal as regards its moisture content.* A review of the literature on the subject shows the following general results:

Greig Smith[1] does not report any experiments upon the inoculation of sterile sugar. Dodson[2] reports positive results from his experiments, but the incubation period was two and a half years' duration, and the moisture content of the sugar is, unfortunately, not reported. Brain and Deerr[3] obtained positive results from their bacterial inoculations, but the moisture content of their sugars varied from a minimum of approximately 2.2 to a maximum of over 4 per cent. As compared with a normal Hawaiian 96-test sugar, it is apparent that their inoculated

[1] Loc. cit.
[2] La. Bul. No. 75.
[3] Loc. cit.

sugars carried an excessive amount of moisture. Owen[1] in a previous publication reported results from inoculations. Although the moisture content of the 96-test sugars used was in most cases between 2 and 3 per cent, the deterioration during an incubation period of about three months was very small and entirely negligible in many instances.

If we take the limitation of density into account in explaining the spheres of activity of bacteria in different types of sugar, then we must also apply the same limitation in explaining the limit to which this action can extend. If the molasses film surrounding the sugar crystal must be comparatively dilute to enable the bacteria to destroy sucrose, then the film must contain a correspondingly small amount of sucrose. Nor is it probable that the conditions resulting from the destruction of this sucrose would be conducive to a further dissolving of the sugar crystal. On the contrary, since the dissolved sucrose is transformed by the bacteria into gum and reducing sugar, the solvent action of the film moisture would more likely be decreased rather than increased. This decreased solubility of the sugar crystal in the surrounding film resulting from the formation of solids non sucrose by bacterial action appears even more probable in the light of another theory. Thus Browne[2] mentions as a possibility that these solids non sucrose in the molasses film may seal off the sugar crystal from the film and thus prevent the latter from taking up more sucrose.

SUMMARY OF PART II, A.

(1) The gum forming bacteria which occur in sugars may be regarded as derived types of the potato group of bacteria. The majority of the cultures investigated seem more closely related in morphological and physiological characteristics to *Bac. vulgatus* than to the other members of the group.

(2) *Bac. vulgatus* and *Bac. mes. ruber* when isolated from sources other than sugar products have a comparatively low sucrose destroying power. This power can be greatly increased by cultivating these species for successive generations in a nutrient sucrose solution. Having acquired the power, on a

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[1] La. Bul. No. 125.
[2] La. Planter, Vol. LIV, No. 18, 1907, p. 28.

10% sucrose solution, they seem capable of exercising it to a corresponding extent upon a sucrose solution of 34 Brix. This acquired power does not appear to be easily lost when the cultures are cultivated on sugar free media.

(3) The limit of sucrose concentration in which the cultures seem capable of inducing any appreciable action is between 46 and 55 Brix. While there is a difference in time required by the spores and vegetative cells of each culture to develop in sucrose solutions, this difference is not proportionate to the density of the solution.

(4) The cultures of bacteria from sugars have a low tolerance of acid and alkali when grown in sucrose solutions.

(5) The comparatively low maximum density in which these bacteria are capable of developing, suggests that their deteriorative action upon sugars is largely confined to fairly moist sugars, in which the molasses films have been much diluted by the absorption, or addition of moisture.

PART II B.

TORULA.

While investigating[1] the comparative value of various culture media for the quantitative bacteriological analysis of sugars, it was found that the use of the ordinary 10% sucrose agar prevented a very important group of micro-organisms in sugars from developing on the plates. The use of sucrose media of higher density resulted in the isolation of a fairly large number of these cultures. It showed, furthermore, that most sugars contain a larger number of this group of micro-organisms than of bacteria. The occurrence of torula in sugar products has been noted by many investigators, but their action in that environment has not up to this time been closely studied. Jörgensen[2] reports the presence of these forms in sugar factories, and even in the finished sugar. Both Laxa[3] and Schone[4] reported the predominance of torula in plates made from beet juice taken from the diffusion batteries at 30°C.

[1] Bulletin 146, La. Exp. Station.
[2] Micro-organisms and Fermentation, p. 385.
[3] Vereins Zeitschr 1901, Bd. 51.
[4] Bohm Zeitschr 1901, Bd. 26.

Torula have also been observed in condensed milk and are regarded[1] by some as the chief cause of the fermentation which sometimes takes place in these products, causing considerable losses to the manufacturers. The association of these forms with diseased beers is well known. Edwards[2] has made an extensive study of the role played by torula in the production of flavors in cheese. In the cane sugar industry their action is not only the occasion of concern in the deterioration of sugars, but also in the fermentation of table syrups.

For the most extensive study of torula now extant, we are indebted to Will[3]. His investigations of the torula occurring in breweries are very comprehensive, giving a complete morphological description of the species during their cultivation on liquid and solid media. He also records the action of the species with the different sugars, their production of acids and their behavior in competition with culture yeast, etc.

The isolation of torula from sugars was accomplished by the inoculation of a 35 Brix agar with small portions of sugar samples. Their growth in this medium is somewhat slow, but the colonies attain macroscopic dimensions in from two to three days. The sugar agar colonies of the different cultures show considerable distinctiveness. Seventeen distinctive cultures were obtained in the course of the investigation.

The following are the principal morphological and cultural characteristics of the cultures:

CULTURE I.

Cells spherical or slightly elliptical in shape; weakly stained with Grams iodine solution; cells varying in diameter from 3-7M. No spores observed.

On potatoes the growth is grayish white; and dry, and confined to within one-eighth of an inch of line of smear.

On 50% sucrose agar slants, the growth is brown and smooth.

On molasses agar the growth is smooth raised, and very dark in color. Fifty per cent sucrose gelatin not liquefied in two weeks.

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[1] Lafar Handbuch der Technischen-Mykologie, Bd. II, 292.

[2] Fruity, or sweet flavor in cheddar cheese. Cent fur Bakt. Abt. II 1913, Bd. 39

[3] Cent fur Bakt. Abt. II 1907, 1908, Bd. 39, 450.

CULTURE II.

In shape and size similar to Culture I; spherical cells vary from 3.4-6.2M in diameter. No spores observed; not stained with iodine. On potato slants a more abundant growth than with Culture I. The growth is most raised on edges, and of a creamy color. On 50% sucrose agar slants the growth is smooth, and white. On molasses agar as in Culture I.

CULTURE III.

Cells shorter and smaller in diameter than the above culture. No spores observed. Cells not stained with iodine solution. On potato slants the growth is smooth, flat and spreading. On 50% sucrose agar the growth is smooth and flat. On molasses agar slants the growth is smooth and flat.

CULTURE IV.

Small round cells, varying in diameter from 3-4M; no spores observed.

CULTURE VI.

Cells round and elliptical, varying in size from 4 to 9M. Weakly stained with iodine solution. No spores observed.

On potato slant the growth is moist white and glistening, and more luxuriant than the previous culture.

On agar slants like culture previously described.

On molasses agar slants, the growth is smooth and very dark.

CULTURE VIII.

Very small cells, from 3-5M diameter. On potato the growth is white, flat and spreading. No spores observed.

On 50% sucrose agar the growth is smooth raised, and light brown in color.

On molasses agar slants the growth is smooth and raised, and very luxuriant.

CULTURE IX.

Small cells as in No. VIII, but more elliptical. Length 5M. No spores observed.

On potato the growth is very feeble, and barely discernible after two weeks' incubation.

On 50% sucrose agar the growth is raised and luxuriant.

On molasses agar slant the growth is filamentous, like Culture IV, except that it is much darker in color.

CULTURE X.

Small cells resembling the lemon-shaped cells of *Saccharomyces apiculatus*. Cells weakly stained with iodine. On sucrose agar the growth is slow. No spores observed.

On potato slants the growth is more abundant. The culture forms a yellowish spreading growth on the entire upper surface. On molasses agar slants the growth is similar to Culture IX.

CULTURE XI.

In both shape and size of cells this culture varies from the ones previously described. The cells are very large, and very unsymmetrical in shape. No spores observed.

On potato slant the growth is moist and raised, and grayish white in color.

On molasses agar slants the growth is similar to Culture IX.

CULTURE XII.

Small elliptical cells as in No. X. On potato slants the growth is white and dry. The cells are more deeply stained with iodine than was true of No. X.

On molasses agar the growth is smooth and very black.

CULTURE XIII.

In size and shape like Culture XII. Cells very weakly stained with iodine. Colonies on sucrose agar very small and round. On potato slants the growth is very feeble.

On molasses agar the growth is very black, but filamentous like Culture IX.

CULTURE XIV.

Cells larger and more deeply stained with iodine than Culture XIII.

On potato the culture resembles Culture XIII. On sucrose agar the growth is much more luxuriant than in the preceding culture.

On molasses agar the growth is very similar to Culture XIII.

CULTURE XV.

Very small spherical cells, giving a very pronounced glycogen reaction with iodine.

On potato slants the growth is very luxuriant; gray growth; edges of growth irregular.

On sucrose agar the colonies are very small, and only slightly raised.

On molasses agar slants, smooth brown growth.

CULTURE XVI.

Cells darkly stained as above. More elliptical in shape, and larger in size.

On potato slants there is a very luxuriant dry growth.

The colonies on sucrose agar are larger and more irregular than from previous cultures.

CULTURE XVII.

The cells are very long and narrow. Stain very deeply with iodine. Cells are grouped in characteristic bunches. Very distinctive in cell grouping.

On potato slants, the growth is very feeble and forms an almost transparent veil over the surface of the potato.

On molasses agar the growth is very dark and smooth.

Schizosaccharomyces in Sugars.

In the course of our investigations of the yeastlike microorganisms in sugars, we encountered two cultures of spore-forming fission yeast, both of which seem closely related to *Saccharomyces octosporus* (Beijerinck)[1].

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[1] Cent. fur Bakt. XVI, 1894. For a complete detailed description of this species the reade- is referred to Schionning's excellent work, a resume of which is given in Jorgensen's micro organisms and Fermentation, pp. 377-381.

CULTURE VII.

This culture resembles No. V, but the cells are not as much constricted in the center. Cultural features agree with those of No. V. This culture also forms endogenous spores, which are usually four in number.

ADDITIONAL FEATURES.

Most of Will's cultures of torula liquefied gelatine. Only a few of those found in sugars were capable of liquefying a 25% sucrose gelatine.

The cultures do not coagulate milk, although they produce various characteristic odors in it. This is in agreement with Will's observations, as is also the difference in the glycogen reaction of the various cultures. The question of whether the cultures do or do not form spores was tested on gypsum blocks, as well as on potato slants, both methods being well calculated to demonstrate the possession or the lack of this ability on the part of these micro-organisms.

The wide scope of this investigation of sugar deterioration rendered impossible a more detailed study of the morphological and physiological characteristics of this interesting group of micro-organisms. The author is free to confess that the data here presented on that subject leaves much to be desired so far as regards the finer and more delicate differentiating characteristics of the torula in cane sugars.

For the present, we must concern ourselves more with the action of these cultures upon sugar products than with the elaboration of more refined methods of differentiating the species.

The occurrence of Saccharomyces octosporous-like species in cane sugar is of especial interest in view of the fact that a patent for its use as a means of recovering sucrose from exhausted cane molasses was granted to Glashan[1]. For the same purpose Pellet and Pairault[2] believed they had isolated an even more suitable yeast.

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[1] Lafar Handbuch der Technischen Mykologie, Bd. II, p. 500.

Bul. Assoc. Chim. de Sucr, et de dist, 1905, Bd. 23, s639.

[3] For a very clear explanation of the impracticability of this process, the reader should consult Brown's Handbook of Sugar Analysis, p. 651.

Culture V isolated from Cuban sugar (cells varying in shape from spherical to characteristic dumbbell forms). In size from 5-8M and 16M in length. The cells lengthen before fission, and again coalesce so as to form the elliptical dumbbell form.

This culture appeared to form only four spores, which is frequently the case with the *Saccharomyces octosporous.*

On potato the growth is moist, flat and white, spreading over the entire surface of the potato.

On molasses agar the growth is filamentous in form, and brown in color.

ACTION OF TORULA CULTURES UPON SUGAR SOLUTIONS.

In the following table is given the results of an experiment upon the action of the torula cultures upon a 50% sucrose solution, made according to Smith's formula. The period of incubation was one month.

TABLE I.

Experiment upon the Action of Yeast Cultures obtained from Sugars, upon 50% Sucrose Solution. (Smith's formula).
Incubation 1 month.
Inoculated from Agar tubes.

Culture	Total Solids Brix	R. S.	Sucrose	
			S. P.	S. C.
Control	51.40	.06	50.70	51.20
Yeast Culture I	51.68	.09	50.55	50.93
" II	51.23	.35	49.10	49.61
" III	50.73	.10	50.10	50.37
" IV	50.73	.35	49.60	49.90
" V	50.03	.28	47.90	49.18
" VI	47.75	8.33	36.20	35.95
" VII	51.15	.03	49.85	51.13
" VIII	51.15	.10	49.95	50.35
" IX	50.65	.08	49.55	49.90
" X	50.65	.41	49.40	49.57
" XI	51.68	.08	50.40	50.60
" XII	50.20	.40	48.05	Lost
" XIII	51.85	.39	48.85	49.39
" XIV	51.85	.33	48.75	48.62
" XV	46.22	1.56	39.75	40.61
" XVI	50.12	4.17	41.00	42.26
" XVII	52.43	.13	49.80	50.38

From the above table it will be seen that in most cases the destruction of sucrose by these cultures was very small. It was highest in Culture VI and Culture XV, but even then it was very weak as compared with that of the bacterial cultures from sugars, when grown in a solution of the same composition. It will be noted that in most of the flasks the amount of invert sugar, at the end of the experiment, was little less than one-half of the amount of sucrose destroyed. In Will's investigation of the torula in breweries, he found that the different cultures varied greatly in their power to invert sucrose. Many lacked this property entirely, others possessed it in degrees varying from very weak to fairly strong. As regards the ability of his cultures to ferment dextrose and levulose, he found that in most cases both of these sugars were utilized to an equal degree. In some cases, however, dextrose was more readily attacked, and in others levulose. The degree of the selective action, however, was never very pronounced.

ACTION OF THE CULTURE IN 50% DEXTROSE AND SIMILAR SOLUTIONS.

TABLE II.

	Levulose	Dextrose
Control	50%	50%
Control	50	50
I Cult.	49.50	48.78
II “	49.02	47.39
III “	47.61	49.02
IV “	50.00	49.50
V “	48.07	49.02
VI “	50.00	47.39
VII “	48.78	48.78
VIII “	47.61	46.51
IX “	49.50	48.78
X “	48.78	48.07
XI “	47.17	49.02
XII “	49.02	47.61
XIII “	48.78	49.02
XIV “	47.50	47.61
XV “	48.54	48.54
XVI “	49.02	48.78
XVII “	48.78	48.54

LEVULOSE—DEXTROSE.

It will be noted from the above table that there seems to be no decided selective action of the cultures of torula for either of the two sugars. Both dextrose and levulose seemed to have been utilized with equal facility by each culture. The amount of fermentation was comparatively small in all cases.

ACTION OF TORULA CULTURES UPON DILUTED FINAL MOLASSES.

A final molasses was diluted to 40 Brix and 200 cc portions poured into flasks and sterilized by the intermittent method. The flasks were then inoculated from molasses agar cultures of the torula.

TABLE III.

Experiment On the Action of Yeasts Isolated from Sugars Upon Half Dilution Molasses.
One month's incubation period. Inoculated from agar tubes of half dilution molasses.

Cult.	Total Solids	Reducing Sugars	Sucrose Direct Polarization	Sucrose Clerget	Total Sugars Loss	
Control I............	40.70	18.3	9.10	12.28	30.58	....
“ II............	38.70	18 3	9.6	11.69	29.97	....
Average Control......	39.70	18.3	9.35	11.98	30.27	....
Yeast Cult. I.........	34.30	19.0	5.6	3.81	7.46	....
“ II.........	33.75	18.0	6.0	4.39	3.88	....
Cult. III............	34.30	18.3	7.4	5.41	6.56	....
“ IV............	34.85	20.2	7.4	5.07	5.00	....
“ V............	34.74	20.2	9.4	4.13	6.12	....
“ VI............	33.66	17.6	10.0	4.08	8.59	....
“ VII............	36.91	18.3	7.6	4.51	2.41	....
“ VIII............	35.81	20.2	7.3	3.94	6.13	....
“ IX............	36.91	20.2	8.0	3.89	6.18	....
“ X............	35.90	20.2	9.6	3.42	6.65	....
“ XI............	35.35	20.2	8.4	3.18	6.89	....
“ XII............	29.30	17.5	10.4	3.78	8.99	....
“ XIII............	35.35	20.9	9.5	3.93	5.44	....
“ XIV............	30.40	20.2	9.8	4.50	5.57	....
“ XV............	34.45	17.2	11.0	4.10	8.97	....
“ XVI............	34.45	21.3	8.0	3.56	5 41	....
“ XVII............	35.00	19.6	4.2	3.67	7.00	....
Bact. Cult. I.........	40.30	18.7	10.2	12.15	.....	Av. loss in Sucrose 6.30

The results of the experiment were interesting, since the amount of invert sugar in the inoculated flasks tended to remain constant in spite of the fact that some inversion had taken place. The increase in invert sugar in the inoculated flasks as compared with the control was never more than 2%, while the decrease in sucrose in these flasks was 7-8%. The experiment was repeated, with the results shown in the following table:

TABLE IV.

Half Dilution Molasses Inoculated with Yeast Cultures from Sugar Inoculated October. Analyzed November.

Sample	Inoculated Culture	Total Solids	Reducing Sugars	SUCROSE S. P.	SUCROSE S. C.	Total Sugars	Loss in Total Sugars
1 Control		38.70	12.6	11.0	13.69	26.29	
2							
3	Cult. I	32.92	9.5	2.74	0.61	10.11	14.81
4	" II	32.58	10.6	3.70	1.37	11.97	12.95
5	" III	33.93	11.5	3.34	4.57	16.07	8.85
6	" IV	32.80	14.8	2.22	3.04	17.84	7.08
7	" V	32.80	14.24	4.32	4.31	18.55	6.37
8	" VI	30.52	9.30	8.26	7.66	16.96	7.96
9	" VII	31.63	10.00	8.30	7.35	17.35	7.57
10	" VIII	32.32	10.40	8.20	7.62	18.02	6.90
11	" IX	33.80	14.10	8.00	8.32	22.42	2.50
12	" X	34.38	13.7	7.64	7.72	21.42	3.50
13	" XI	34.08	16.4	7.24	7.97	24.37	0.55
14	" XII	33.83	9.05	9.80	9.40	18.45	6.47
15	" XIII	33.90	12.00	14.16	12.45	24.45	0.47
16	" XIV	34.38	12.56	11.00	9.50	22.06	2.86
17	" XV	34.13	8.70	10.00	8.72	17.42	7.50
18	" XVI	35.28	12.40	12.7	11.18	23.58	1.34
19	" XVII Y. & B.	33.88	10.00	7.65	7.35	17.35	7.57
20	" XVII Y.	34.13	10.8	6.44	7.15	17.95	6.97
21							Av. Loss 6.22

In this experiment the loss in sucrose was higher in some cases than in the previous experiment. There was also a greater tendency for the invert sugar of the inoculated samples to vary from that of the control. This variation took the form of an increase in certain cases and a decrease in others. The limits of the variation seemed to be about 3% either way. In other words, it appeared that in some cases the torula cultures formed more invert sugar than they could ferment during the period of incubation, and in other cases, less. An average of all the inoculation results tends to show a preference on the part of the organisms to ferment only as much invert sugar as it formed from the inversion of the sucrose in the solution. An interesting point in connection with the above table is the fact that in many cases the single polarization of the inoculated samples was higher than the sucrose Clerget. This indicated a selective action of certain of the cultures upon levulose, which resulted in an accumulation of dextrose in the solution.

The fermentation of molasses and syrups by torula often results in the development of various esters. It is to the formation of such products that deteriorating sugars owe their characteristic aroma. The fruity odor of deteriorating sugars has been mentioned by various writers. Thus Browne[1] calls attention to this fact as follows:

> *"The strong odor of esters, which is so perceptible upon entering a warehouse or the hold of a ship where raw sugar is stored, is produced by micro-organisms. This odor is sometimes regarded as an evidence of deterioration, but this is not necessarily the case. Sealed samples of raw sugars may develop an intense rum-like odor without showing the slightest loss in polarizaton."*

We will discontinue the further consideration of this group of micro-organisms, but will again advert to it in a subsequent review of the comparative action of the various groups of micro-organisms upon cane sugars and cane sugar products.

PART II C.

MOULDS.

As far as can be learned from the fragmentary literature on the subject of sugar deterioration, it appears that it was Shorey who first attributed such changes in the composition of sugars during storage to the activities of moulds. In a paper,[2] presented in 1898 at the May meeting of the Society of Chemical Industry, the author above referred to stated that it is *Penicillium glaucum,* and not bacteria, that are responsible for the deterioration of raw cane sugars. Shorey's work, while highly valuable, was not sufficiently complete, and it could not, therefore, have been expected to have resulted in a prompt and general acceptance of his theory. His theory was founded on the following observations:

(1) The presence of *Penicillium glaucum* in sugars.
(2) The deterioration of alkaline sugars and the ability of *Penicillium glaucum* to invert sucrose solutions of a similar degree of alkalinity.
(3) The development of *Penicillium glaucum* in solutions made from deteriorating sugars.

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[1] Chemical Factors in the Deterioration of Raw Sugars.

[2] The Deterioration of Raw Cane Sugar in Transit or Storage. The Journal of the Society of Chemical Industry, June, 1898.

While these deductions might very probably have been the true explanation of one of the causes of sugar deterioration, yet the experimental data presented did not eliminate other causes, which might also have been operative. Shorey himself claimed for his deductions an application only to the special cases which had come under his observation.

The fact that subsequent investigators did not build upon the foundation which Shorey had laid was due to the following causes, viz.: (1) The predominance of bacteria developing upon plates made from sugars, which was due to the prevalent use of a medium especially favorable to that class of micro-organisms. (2) The incompleteness of Shorey's data.

Thus we find Dodson,[1] Greig Smith,[2] Deerr,[3] Norris,[4], Brain[5] and Owen[6] leaving moulds out of consideration as one of the causative agencies in sugar deterioration.

It is true that Deerr and Norris[7] admitted that in certain cases the numbers of bacteria in some of the samples of deteriorating sugar which came under their observation were too low to account for the chemical changes which occurred. They did not, however, attribute these changes to any special group of micro-organisms. In connection with the study of the deterioration of beet sugars, Schone[8] mentions the occurrence in these products of *Penicillium glaucum,* and also, but much less frequently, aspergillus and mucor species. He did not credit them, however, with any definite connection with the deterioration of sugars.

The secretion of invertase by species of the aspergillus, penicillium and other groups of fungi has long been known. As early as 1878 Gayon[9] found that aspergillus niger formed invert sugar when grown in a medium containing cane sugar. This discovery was confirmed by Duclaux[10] in 1883. The isolation of invertase from this species of mould was successfully carried out for the first time by Fernbach in 1890. The isolation of invertase from

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[1] Loc. cit.
[2] Loc. cit.
[3] Loc. cit.
[4] Loc. cit.
[5] Loc. cit.
[6] Loc. cit.
[7] Loc. cit.
[8] Latar Handbuch der Technischen Mykologie Bd. II.
[9] [10] Soluble Ferments and Fermentation, p. 121. J. Reynolds Green.

Penicillium glaucum was effected by Bourquelot in 1886. The limits of density, however, in which these species are able to invert sucrose does not seem to have been thoroughly studied.

THE ISOLATION OF MOULDS FROM SUGARS.

Sugar samples were transferred to sterile 50% sucrose solution tubes. The tubes were then placed in the incubator and kept for several days at 34°C. When mould growth appeared a transfer was made to tubes of sterile agar, which were poured into plates. A similar series was made from all of the sugar samples on hand, using a molasses agar instead of the sucrose agar. Any decided difference in the appearance of the moulds developing upon the plates, made as above described, was deemed sufficient ground to regard the different growths as distinct species. This method of classification applied, of course, only to the use of the cultures in the preliminary experiments. All of the cultures were thereafter submitted to Dr. Charles Thom, of the Microbiological Laboratory of the Bureau of Chemistry, who very kindly offered to identify the cultures for us. The data on these mould cultures furnished us by Dr. Thom and the microanalyst of his department, Miss Margaret Church, are so valuable that it is deemed advisable to embody their reports in the text of this publication. The following are the data as transcribed from their respective letters:

> *Cultures I, IV and IX are Aspergillus niger.*
>
> *Culture II is Aspergillus fumigatus.*
>
> *Culture III is related to Aspergillus glaucus.*
>
> *Culture VI is a blue Aspergillus.*
>
> *Cultures VII and VIII are either floccose Aspergillus niger strain or else contaminated with some mould at present unrecognizable by us.*
>
> *Cultures X and XIII are black or brown forms belonging to the Aspergillus niger group.*
>
> *Culture XI is a blue-green form which we have gotten from several sources but have not satisfied ourselves as to name.*
>
> *Culture XII is a sterile organism of orange color which we find constantly. Further than this, I know nothing*

about it, since no fruiting has ever occurred, in spite of various attempts to induce it.

Culture XIV belongs to the Aspergillus glaucus series, probably being closest to A. repens.

The Culture III proved to consist of two organisms, a black and a green one. The green one proved to be a very interesting culture, which we have tentatively identified as Aspergillus pencilloides of Spegazzini. This was described by Spegazzini in a series of cultures obtained from sugar cane, in Argentina, some thirty years ago, and furnished a lot of interesting but troublesome data.

The authors suggest an interesting subject for investigation in connection with the inverting power of these mould cultures. The suggestion is as follows: *"If you could keep Nos. VII and VIII in a floccose and also in a purely fruiting condition during a series of transfers, and could establish a difference in the inverting power of either of these strains of Aspergillus niger according to the excess of mycelium or conidia which the cultures tended to produce, we would be decidedly interested."*

It is to be regretted that the lack of time for the further prosecution of this investigation prevented us from acting upon this valuable suggestion.

Referring to the description of the mould cultures, it will be noted that there were more duplications submitted of No. 1 than of any of the other cultures. That, of course, indicates the greater frequency with which this culture is found to occur in sugars, as compared with the other cultures.

What has been said with reference to the distribution of the bacterial cultures in sugars applies to an equal extent to the mould cultures. In many sugars, almost all of these cultures can be isolated. The difference in the infection of sugars with moulds seems also to be one of degree and not of kind. Sugars are rarely found to be entirely free from mould spores. It will be noted that the list of cultures obtained from sugars does not include a single one of *Penicillium glaucum.* This appears strange in view of Shorey's observation, as well as the general impression regarding the frequent occurrence of the mould in sugar products. A green mould is very frequently found on the culture plates made from sugars, and for a long time the author regarded it as *Penicillium glaucum.* A study of its mode of spore formation, however, showed that it belonged to the aspergillus rather than to the penicillium group.

ACTION OF THE MOULD CULTURE UPON SUGAR SOLUTIONS.

The ability of any group of micro-organisms to cause the deterioration of sugars must depend at least upon the one condition that they can develop and can exercise this ability in highly concentrated sucrose solutions. To demonstrate this ability on the part of the mould cultures, the following experiments were conducted: Several series of solutions were made from a Cuban 96-test sugar. The solution varied in density from 52 to 72 Brix. Two hundred cc portions of these solutions were introduced into 300 cc Erlenmeyer flasks. The flasks and contents were then sterilized on three successive days and then inoculated with spores of the different cultures.

TABLE I.

Experiment III.
Table Showing Results of Experiment upon the Limits of Density in which Mould Cultures can Invert Sucrose.
One month Incubation.

Series	Cult No.	Name	Corrected Brix	Sucrose		% R. S.	Acidity 10 gm. Req. cc N/10	Loss in S. C.	Increase in R. S.
				S. P.	S. C.				
A	Cont...	....	52.01	38.2	40.57	12.5	5.	—	—
"	I	Asp. niger...	53.69	1.3	12.3	25.0	–	28.27	12.5
"	II	Asp. Fumigatus...	53.21	21.2	27.78	34.7	1.2	12.79	22.2
"	VI	Blue Aspergillus.......	52.71	33.6	37.67	19.51	1.0	2.9	7.0
"	VII	....	53.21	– .7	5.4	45.9	2.9	35.17	33.4
"	XI	....	52.79	38.2	40.81	11.41	0.5	—	—
"	XIII	....	52.67	4.2	14.25	42.10	2.0	26.32	29.6
"	VIII	Floccose Asp. niger...	53.08	0.9	17.5	44.4	2.2	23.07	31.9
"	XIV	....	52.83	20.0	26 7	26.6	0.6	13.87	14.1

Experiment III---Continued.

TABLE II.

MOULD CULTURES

Series	Culture No.	Corrected Brix	% R. S.	Sucrose		Acidity 10grm. cc Req. N/10	Loss in S. C.	Increase in R. S.	B. — S. C.
				S. P.	S. C.				
B	Cont.......	61.08	12.1	51.5	53.39	0.5	—	—	7.69
"	I	62.55	34.7	19.1	28.19	2.8	25.20	22.58	27.85
"	II	61.26	16.0	41.2	45.27	0.9	8.12	3.88	15.99
"	VI	61.39	14.03	46.25	49.23	0.8	4.16	1.91	12.16
"	VII	61.39	43.10	17.9	26.97	2.1	26.42	29.98	34.42
"	VIII	61.98	42.10	17.0	26.63	2.0	27.36	29.98	35.35
"	XI	61.33	6.7	53.0	54.46	0.5	—	—	6.87
"	XIII	61.98	36.3	19.2	28.04	2.1	25.35	24.18	33.94
"	XIV	61.33	27.5	30.0	36.57	0.7	16.82	15.32	33.83

Experiment No. III.

TABLE III.

MOULD CULTURES

Series	Culture No.	Corrected Brix	Sucrose		% R. S.	Acidity 10gm. — cc N/10 Req.	Decrease in Sucrose	Increase in R. S.
			S. P.	S. C.				
C	Mould I.............	68.80	43.95	41.39	14.08	2.1	16.22	12.03
	" II............	69.20	51.20	47.23	6.09	2.9	11.38	4.04
"	" VI...........	68.83	64.50	57.61	2.51	0.9	1.00	0.46
"	" VII..........	69.00	52.70	48.35	12.60	2.8	10.26	10.55
"	" VIII.........	68.80	49.60	45.91	14.40	3.5	12.70	12.35
"	" XI...........	68.85	65.45	58.47	2.20	0.8	0.14	0.15
"	" XII..........	69.00	65.80	58.66	2.10	1.0	—	0.05
"	" XIII.........	68.83	50.30	46.76	12.30	3.3	11.85	10.25
"	" XIV..........	69.63	9.10	13.92	29.40	1.0	44.69	27.35
"	Mould VII—........ Yeast I —	67.35	63.60	57.42	0.29	2.2	1.19	—
"	Yeast...............	67.55	65.50	58.61	0.28	2.0	—	—
"	Control.............	68.75	65.40	58.61	2.05	0.9	—	—

Experiment III---Continued.

TABLE IV.

MOULD CULTURES

Series	Culture No.	Corrected Brix	Sucrose		% R. S.	Acidity 10gm. — cc N/10 Req.	Loss in Sucrose	Increase in R. S.
			S. P.	S. C.				
D	I...............	72.26	36.9	40.95	15.73	—	0.39	0.49
"	II..............	71.06	37.0	41.03	15.73	—	0.31	0.49
"	VI..............	72.02	37.2	41.10	15.73	—	0.24	0.49
"	VII.............	71.52	37.0	40.95	15.73	—	0.39	0.49
"	VIII............	72.26	37.0	41.03	15.24	—	0.31	—
"	XIV.............	71.76	37.8	41.57	14.78	—	—	—
"	Control............	72.50	37.3	41.34	15.24	—	—	—

RESULTS OF THE EXPERIMENTS.

A study of the above tables shows that with the exception of Culture XI all the cultures exercised a very strong inverting action in the solution below 72 Brix density. No inversion at all took place in that series, which indicates that the limit for inversion by these mould species is below that density. Culture X exercised no inverting action in any of the series. Cultures VII and VIII exercised the strongest inverting action of any of the cultures. The amount of inversion of sucrose in the sugar solution of 68 Brix is astounding, and indicates an ability on the part of these moulds to exercise a decided sucrose destroying power in the molasses films of certain types of sugars. If we study the results of Tables I and II we notice an interesting point in regard to the comparison between the amount of sucrose inverted and the increase in reducing sugar. In many cases we find that the increase in the latter is much greater than can be accounted for by the decrease in sucrose. Note, for example, Nos. VII and VIII in Tables I and II. This is possibly due to the formation of some reducing substance in addition to reducing sugars.

In connection with the rapid inversion by moulds of sucrose in solution of 69 Brix, it is interesting to note that the action of invertase is supposed to be very feeble in that concentration. O'Sullivan and Thompson[1] found that the rate of action of invertase decreased only slightly from an optimum concentration of 20 until 40% was reached. Beyond that point it was greatly enfeebled. Bokorny,[2] in working with compressed yeast, found that although these yeast could induce a vigorous fermentation in glucose solutions of 58.8%, yet in a similar concentration of cane sugar no fermentation took place. Only when a concentration of 74% was reached was the yeast inactive towards the former sugar. Bokorny would explain the suppression of invertase action in concentrated sucrose solution by attributing it to the lack of water necessary for the hydrolysis.

It seems evident that the invertase of moulds differs from that of yeast in its ability to retain its activity under conditions of high concentration.

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[1] Jour. Chem. Soc. Trans. 57, 1890, 834.

[2] Beinflussing des Hefie Invertins durch Konzentrierte Zuckerlosungen Chem. Ztg. 1903, No. 90.

ARE TORULA AND MOULDS COMPETITIVE OR SYMBIOTIC IN THEIR ASSOCIATION IN SUGARS?

In a previous section of this report it was stated that although the torula in sugars could only invert sucrose in solutions of concentration of about 60 Brix, they are able to ferment glucose in solutions of very much greater density. This fact appears to offer a possibility for the deterioration of very high density sugar solutions by the combined activities of torula and mould. The possibility of this combined action depends, of course, upon whether these two groups of micro-organisms are competitive or symbiotic in their association. Referring to Table III we find that while the combined inoculation of Culture VII mould, and Culture I yeast, resulted in a slight loss of sucrose, this loss was much lower than that produced by the mould alone. In this case it appears that the activities of the yeast had greatly retarded the sucrose destroying power of the mould.

A similar experiment was next made upon a sugar solution of 71.5 Brix.

TABLE V.
Table Showing Action of Yeast and Mould Cultures on 71 Brix Molasses.
7-19-17

Samples	Treatment	Total Solids Brix	Sucrose		% R. S.	Acidity 10 gm. Req. cc
			S. P.	S. C.		
I	Control	71.58	37.2	40.76	14.4	9.50
II	Yeast Culture I	68.83	46.0	43.16	7.75	10.50
III	Mould II Yeast I	68.40	45.6	42.24	7.60	9.50
IV	Mould I Yeast I	69.15	46.4	43.47	7.54	9.00
V	Mould VII Yeast I	68.20	46.0	43.07	8.09	10.00
VI	Mould VIII Yeast I	68.60	45.6	43.38	8.63	10.50
VII	Mould VI Yeast I	68.80	45.6	43.07	10.26	9.00

In the above table it will be seen that the action of the yeast in reducing the amount of total solids in the solution did not enable the mould cultures to invert any sucrose. On the contrary, the increase in purity of the inoculated solution was just as characteristic as if the torula had been used alone. Evidently the decrease in density of the solution brought about by the torula affords no advantage to the mould, but seems rather to suppress the activities of the latter by the accumulation of objectionable fermentation products.

PART II D.

THE CONDITIONS LIMITING THE RESPECTIVE ACTIVITIES OF THE THREE GROUPS OF MICRO-ORGANISMS IN SUGARS.

It is natural to suppose that the variation in the composition of sugars of different types would represent conditions of unequal suitability for the development and activities of the different groups of micro-organisms occurring therein. Under what conditions does the one or the other group gain the ascendancy? It is to the answering of this question that we will now direct our attention. From a superficial consideration of the subject, we might well conclude that the conditions differentiating the possible activities of the three groups of micro-organisms in sugars might be classified as follows:

(1) Degree of density of the molasses film.
(2) Its nutritive value.
(3) Its degree of acidity or alkalinity.

Let us consider these in their relative order. A series of solutions was made from 96-test sugar. The solutions varied in density from 37 to 72 Brix. The solutions were sterilized and inoculated with a culture of bacteria and a yeast, and also from a combination of the two. The results are given in the following table:

TABLE I.

Experiment on the Influence of Concentration of Sugars, upon the Differentiation of Action of Bacteria and Yeast.
Solutions made from 96-Test Sugar. One month Incubation.

Series	Treatment	Sucrose		Brix	R. S.	Loss in S. C.	Increase in R. S.	Decrease in R. S.
		S. P.	S. C.					
A 37 Brix	Cont.	34.4	34.98	37.31	1.58	—	—	—
	Bact.	– 6.6	2.69	38.52	29.46	32.29	27.88	......
	Yeast	33.75	33.63	35.27	>.40	1.35	—	1.18
	Combined	4.6	6.96	35.72	19.50	28.02	17.92	—
B 43 Brix	Cont.	39.70	39.90	43.34	2.15	—	—	—
	Bact.	– 5.80	3.90	43.23	39.90	.36	37.75	—
	Yeast	38.20	38.11	40.60	>.84	1.79	—	1.31
	Combined	28.20	29.23	37.53	3.79	10.61	1.64	—
C 52 Brix	Cont.	48.45	48.73	52.73	2.78	—	—	—
	Bact.	–12.95	1.84	54.18	25.00	46.89	22.22	—
	Yeast	39.50	38.70	48.40	5.12	10.03	2.34	—
	Combined	37.45	36.83	47.45	5.65	11.90	2.87	—
D 60 Brix	Cont.	55.80	55.94	60.83	3.57	—	—	—
	Bact.	55.85	56.06	61.25	3.57	—	—	—
	Yeast	42.90	43.78	55.80	7.69	12.16	4.12	—
	Combined	41.45	42.09	55.55	7.89	13.85	4.32	—
E 72 Brix	Cont.	66.30	67.31	72.30	4.54	—	—	—
	Bact.	66.40	67.46	72.54	4.54	—	—	—
	Yeast	67.45	67.70	69.84	0.73	—	—	3.81
	Combined	67.75	68.10	70.69	0.69	—	—	3.85

From this table it will be seen that the destruction of sucrose by the bacterial cultures ended with the series C (52 Brix). The torula culture caused deterioration in the series D (60 Brix). Only in one case did the combined inoculation result in a greater destruction of sucrose than either of the other inoculations, and that was in series D. In the series in which the bacteria exercised any action the combined inoculation decreased the amount of sucrose destroyed, as compared with the solutions inoculated with bacteria alone. It is interesting to note that in both Series A and B the inoculation with yeast alone resulted in a decrease of reducing sugar similar to that which took place in higher density solutions. In fact, the results in these series are very similar to those of Series E. It is probable that the difference in the acidity of the solutions was responsible for these results. In the more dilute solutions less sugar was used, and the acidity of the solution was correspondingly lower than in the higher density solutions.

In the following table are the results of a similar experiment upon the comparative activities of a culture of bacteria and mould. The solutions were prepared and treated as in the previous experiment. The incubation period was two months.

TABLE II.

Experiment upon the Limit of Density in which Bacteria and Mould cause Sugar Deterioration.
Incubation two months.

Series	Treatment	Total Solids Brix	Sucrose		% R. S.	Acidity 10 cc req. N/5 X. 969
			S. P.	S. C.		
A 50 Brix.........	Cont...............	54.42	47.00	48.75	5.88	1.5
	Bact...............	55.50	46.90	48.53	5.88	—
	Mould............	55.50	−14.40	3.69	50.00	12.5
B 60 Brix.........	Cont...............	64.08	55.50	57.48	6.66	0.5
	Bact...............	63.53	54.90	56.64	6.66	—
	Mould............	64.50	− 6.15	10.20	55.50	3.0
C 65 Brix.........	Cont...............	67.05	57.85	59.45	6.40	0.5
	Bact...............	67.09	58.25	59.89	6.66	—
	Mould............	67.50	2.95	18.40	48.70	3.0
D 70 Brix.........	Cont...............	69.34	61.10	62.74	5.26	0.4
	Bact...............	70.36	61.70	63.32	5.12	—
	Mould............	69.56	9.90	24.48	46.50	3.5

In the above table it will be seen that the inoculation with the mould culture resulted in a marked deterioration of the Series D solution (69 Brix). No deterioration appeared to result from the use of the bacterial culture, even in Series A (55 Brix).

A third experiment was made, using white sugar syrups obtained from three plantations in the State, instead of solutions of 96-test sugar. This experiment was really intended to demonstrate the nutritive demands of the micro-organisms as well as the respective limits of density in which they could destroy sucrose. The syrups were sterilized and inoculated as in the previous experiment.

TABLE III.

Experiment upon the Relative Susceptibility to Deterioration of White Sugar Syrups from various Plantations.
Two months Incubation.

Series	Treatment	Total Solids Brix	Sucrose		% R. S.	Acidity 10 cc req. N/5 X. 969
			S. P.	S. C.		
A—Reserve	Cont.	63.93	50.50	52.59	6.88	1.4
	Bact.	64.26	50.50	52.49	7.14	1.5
	Yeast	58.28	42.30	44.28	5.12	2.4
	Mould	62.21	− 1.50	9.31	30.70	10.0
B—Wilbert	Cont.	49.81	37.45	39.16	5.79	1.7
	Bact.	51.46	38.80	40.62	5.88	1.6
	Yeast	40.98	− 3.00	2.92	40.00	3.0
	Mould	44.92	− 4.70	0.20	31.25	32.0
C—Ashland	Cont.	60.23	48.00	49.90	5.00	1.6
	Bact.	59.83	48.70	50.52	4.87	1.4
	Yeast	54.27	35.20	37.48	7.69	2.5
	Mould	57.88	− 3.10	6.13	39.21	14.0

The results of the experiment show that the mould culture exercises an immensely greater sucrose destroying power than did the cultures of the other micro-organism. The bacteria caused an appreciable loss of sucrose; the yeast culture caused a considerable loss even in the syrup of 63 Brix density.

From the standpoint of the nutritive value of the different syrups, no conclusion can be drawn, as the solutions deteriorated in accordance with what might have been expected from a consideration of their respective densities. We have, therefore, the following data upon the limits of density in which the respective groups of micro-organisms are able to destroy sucrose:

(1) Mould culture: Rapid deterioration in 69 Brix solution.

(2) Torula culture: Moderate deterioration in 64 Brix solution.

(3) Bacteria culture: Rapid deterioration in 52 Brix solution; no deterioration in 60 Brix solution.

THE RESPECTIVE NUTRITIVE REQUIREMENTS OF THE THREE GROUPS OF MICRO-ORGANISMS.

A solution was made of standard granulated sugar and distilled water. To this solution was added amounts of peptone varying from 0.2% to 1%. Three hundred cc Erlenmeyer flasks were then filled to two-thirds capacity with these solutions and sterilized. The flasks were then inoculated with a culture of bacteria and torula.

TABLE IV.

Experiment on Influence of Nitrogen upon the Action of Torula and Sugar Species. Incubation, one month.

Series	Treatment	Brix	Sucrose		% R. S.
			S. P.	S. C.	
A	Control	54.11	51.50	51.41	1.35
No Peptone	Bact	52.51	51.50	51.57	1.37
	Yeast	51.96	50.00	50.04	1.73
	Bact and Yeast	53.54	50.90	50.63	1.60
B	Control	53.85	51.40	51.50	....
O. 2 Peptone	Bact	53.37	50.00	50.64	....
	Yeast	51.92	48.00	47.78	3.20
	Bact. and Yeast	52.91	47.90	47.70	3.33
C	Control	55.14	51.70	—	2.16
O. 5 Peptone	Bact	53.61	52.00	52.16	1.08
	Yeast	52.51	45.10	45.26	4.44
	Bact. and Yeast	51.68	46.00	46.21	4.21
D	Control	54.69	50.80	51.54	3.01
1% Peptone	Bact	54.57	51.00	—	1.37
	Yeast	53.29	45.00	45.82	3.63
	Bact. and Yeast	52.79	43.05	43.74	5.33

From the above table it will se been that the amount of deterioration produced by the torula culture increased with the addition of peptone, until the addition reached 0.5%. There was no further increase beyond that point. There was no action produced by the culture of bacteria, as the density of the solution was probably beyond the maximum for its action.

In the next experiment a solution was made from plantation granulated sugar and distilled water. To this solution was added various nutritive ingredients. The solution was sterilized and inoculated as in the previous experiment.

TABLE V.

Experiment upon the Influence of Nutrient Salts upon the Deterioration of P. G. in Distilled Water.
Period of Incubation. one month.

Series	Treatment	Brix	Sucrose		% R. S.	Loss in Sucrose S. P.	Increase in R. S.	
			S. P.	S. C.				
Plant G. + Dist. Wt.	Control.....	35.65	34.20	34.48	.909	—	—	—
	Bact.........	37.00	− 1.8	2.55	33.3	32.40	32.391	Mould G.
	Yeast........	35.95	32.80	33.23	2.35	1.40	1.441	—
	Combined B. & Y....	36.20	31.20	31.98	4.00	3.00	3.091	—
Plant G. + O. 5 KCL	Control.....	35.95	33.70	34.25	1.48	—	—	—
	Bact.........	36.30	23.50	—	5.18	10.20	3.70	Gummy
	Yeast........	36.05	32.00	32.76	3.47	1.70	1.99	—
	Combined B. & Y.....	36.30	32.25	32.63	3.12	1.45	1.64	—
Plant G. + O. 2% Na3 PO4	Control.....	Lost	—	—	—	—	—	—
	Bact.........	36.20	17.50	—	17.30	—	—	Gummy
	Yeast........	36.45	35.30	35.81	1.62	—	—	—
	B. & Y......	36.45	31.81	—	5.71	—	—	Gummy
Plant G. + O. 5% Peptone	Control.....	38.05	35.40	35.97	.909	—	—	—
	Bact.........	38.05	2.60	—	40.00	32.80	39.091	Gummy
	Yeast........	34.30	10.90	11.56	23.52	24.50	22.611	—
	B. & Y......	35.15	11.90	12.50	23.52	23.50	22.611	—
Plant G. Complete Formula	Control.....	37.25	35.30	35.49	—	—	—	—
	Bact.........	37.25	4.40	—	33.33	30.90	—	Gummy
	Yeast........	31.04	3.90	—	21.62	31.40	—	—
	B. & Y......	30.15	8.40	7.39	18.18	26.90	—	—
Stand. G. + Dist. Wat.	Control.....	36.75	19.00	22.62	16.66	—	—	—
	Bact.........	36.83	23.00	24.53	12.90	—	—	—
	Yeast........	36.83	22.00	26.89	13.33	—	—	—
	B. & Y......	36.28	19.90	25.06	13.79	—	—	—

In the above table it will be seen that the greatest deterioration produced both by bacteria and torula was in the series containing 0.5% peptone. In the series containing only sugar and distilled water there was a very high inversion of sucrose in the flask inoculated with bacteria. In this case, however, the solution contained a mould growth, due to contamination. The greatest deterioration produced by the torula culture was in the series containing all of the ingredients in the proportion prescribed in Smith's formula, viz:

(.5% Kcl .2% Na_3Po_4 .1% Peptone)

While the addition of these nutrient salts alone enabled the bacteria to cause a rapid destruction of sucrose, it did not produce the same effect upon the torula. Neither the bacteria nor the torula culture produced any appreciable effect upon the solution of standard granulated sugar in distilled water. An interesting point in connection with the above results is the large amount of inversion in the standard granulated control solution.

The solution contained no evidence of any fermentation changes, as it was perfectly clear, and no evidence of any microbial development could be found by microscopical examination. The inversion was due to the heating of the solution incident to sterilization. The solution of plantation granulated sugar did not undergo such an inversion, owing, no doubt, to its higher ash content. These solutions were sterilized by steam, at atmospheric pressure, so the amount of inversion in the control was entirely unexpected. This inverting action, however, is fully discussed by Von Lippmann.[1]

Another and much larger series of standard granulated solution was made, which included a larger number of nutrient additions than was used in the previous experiment. The solutions were prepared and inoculated as in the previous experiments.

TABLE VI.

Experiment upon the Deterioration of Standard Granulated Sugar in Distilled Water Solutions with addition of various Nutritive Compounds.
Incubation one month.

Series	Inoculation	Total Solids Brix	Sucrose		R. S.
			S. P.	S. C.	
A Stand.	Control	36.39	27.35	28.71	7.27
Gran.	Bact.	35.88	29.35	25.81	5.40
Dist.	Yeast	36.14	29.00	30.22	5.12
Water	B. & Y.	36.48	29.35	30.52	5.71
B Stand.	Control	35.34	33.40	33.26	0.938
Gran.	Bact.	34.74	32.60	33.44	1.290
Tap	Yeast	36.39	33.40	33.13	0.966
Water	B. & Y.	35.88	33.50	33.36	0.854
C Dist.	Control	35.84	30.85	31.60	3.63
Water	Bact.	35.62	30.40	31.89	4.23
+ .01	Yeast	36.64	30.00	30.86	5.00
Peptone	B. & Y.	36.22	31.50	32.12	3.27
D Dist.	Control	36.14	34.00	34.15	1.13
Water	Bact.	35.37	22.55	Gum	12.90
+ .1%	Yeast	35.64	33.80	33.59	1.08
Peptone	B. & Y.	36.30	33.70	33.92	1.21
E .01%	Control	36.14	34.85	34.72	0.357
Sodium	Bact.	35.84	34.30	35.07	0.802
Phosphate	Yeast	35.92	34.40	34.99	0.869
	B. & Y.	36.37	34.80	34.04	0.588
F Dist.	Control	36.14	35.20	34.93	0.200
Water + .1%	Bact.	35.62	16.8	Gum	16.60
Sodium	Yeast	36.22	35.40	36.18	—
Phosphate	B. & Y.	36.37	34.85	34.71	0.227

Die [1]Chemie der Zuckerarten, Bd. II, p. 1228.

TABLE VI.

Experiment upon the Deterioration of Standard Granulated Sugar in Distilled Water Solutions with addition of various Nutritive Compounds.
Incubation one month.

Series	Inoculation	Total Solids Brix	Sucrose		R. S.
			S. P.	S. C.	
G .1% Peptone .1% Sodium Phosphate	Control	36.18	35.00	34.78	—
	Bact.	35.98	33.80	Gum	1.08
	Yeast	36.00	33.60	34.60	1.86
	B. & Y.	35.82	33.85	33.78	1.81
H .1% Peptone .1% Phosphate	Control	36.18	35.10	35.07	—
	Bact.	36.02	4.9	Gum	26.3
	Yeast	36.30	35.20	34.93	—
	B. & Y.	35.86	34.65	34.66	Trace
I .1% $CaSO_4$	Control	36.43	35.20	35.07	—
	Bact.	35.98	31.90	Gum	3.07
	Yeast	36.14	35.10	34.86	—
	B. & Y.	35.61	34.80	34.84	0.50
J .1% CaSO4	Control	36.18	35.10	34.84	—
	Bact.	35.98	33.20	Gum	2.66
	Yeast	36.07	35.20	34.93	—
	B. & Y.	36.15	33.00	Gum	2.00
K .1% Sugar Ash	Control	35.88	35.20	35.07	—
	Bact.	35.98	34.80	Gum	0.975
	Yeast	36.37	34.40	34.32	0.625
	B. & Y.	35.90	34.80	34.63	0.487
L .1% Sugar Ash	Control	36.18	35.20	35.07	—
	Bact.	35.18	32.80	Gum	2.94
	Yeast	36.15	35.20	34.93	—
	B. & Y.	35.90	33.30	Gum	2.66
M .1% Peptone + .1% Sugar Ash	Control	36.18	35.10	34.91	—
	Bact.	36.02	.64	Gum	25%
	Yeast	36.15	35.30	35.14	—
	B. & Y.	33.79	21.10	Gum	10.52
N .1% Peptone + .1% $CaSO_4$	Control	36.18	35.10	34.91	—
	Bact.	35.98	9.8	Gum	22.7
	Yeast	36.70	35.30	35.01	—
	B. & Y.	35.65	24.35	Gum	9.52

The results in the above table show that the addition of the ingredients to the standard granulated sugar solution did not result in as much deterioration from the action of the torula culture as in the experiment where plantation granulated sugar was used. On the other hand, the deterioration by the culture of bacteria was in many cases larger than in the preceding experiment. We find the greatest deterioration in Series H, which contained 0.1% peptone and 0.1% sodium phosphate. Series M, containing the same amount of peptone and 0.1% sugar ash, ranked next. Neither the bacteria nor the yeast culture could induce any deterioration in the pure standard granulated solution. It is interesting to compare these results with those obtained in the preceding experiment on plantation granulated sugar. In that experiment the torula culture induced quite a considerable amount of deterioration in the solution of plantation granulated sugar in distilled water. It appears, therefore, that the addition of nutrient ingredients in the above experiment was in no case sufficient to make the nutrient value of the standard granulated solution equal to that of the pure plantation granulated solution for the development of the torula. From this it appears very probable that this question of nutrient value does exercise a considerable influence upon the keeping quality of white plantation sugars.

Another very interesting fact, which is brought out in the above experiment, is the relatively small deterioration produced by the combined inoculation as compared with the single inoculation with bacteria. It appears that in those cases the solutions were so deficient in nutrient value that the presence of the yeast resulted in depriving the bacteria of the necessary amount of food. The deterioration, therefore, is reduced as a result of a division of the nutrient ingredients of the solution. If we compare the amount of the inversion in the control of Series A with that of B, as well as with the other series, we find that even the presence of the mineral matter in the tap water acted as a partial buffer against this hydrolysis. The addition of the mineral matter in the other series, even in quantities as small as 1/100 of one per cent, entirely prevented the inversion of sucrose during the heating process.

In order to determine the influence upon the rate of sucrose destruction, of substances which would be likely to constitute the impurities of plantation granulated sugars, the following experiment was conducted. In this experiment, solutions were made from both plantation and standard granulated sugars and distilled water, as in the preceding experiments. The different series were treated as follows:

A. I. Plantation granulated sugar solution.

A. II. Same as above, with the addition of 1 cc of a 1:1000 molasses solution to each 100 cc of solution.

A. III. With 1 cc of a 1:1000 soil suspension to each 100 cc of solution.

A. IV. With 0.1% sodium phosphate.

Series B standard granulated sugar solution treated as in A.

TABLE VII.

Experiment on the Deterioration of Sugar Solutions from A-Plantation Granulated and Distilled Water.
Three weeks Incubation.

Series A	Inoculation	Corrected Brix	Sucrose		% R. S.	Loss in Sucrose	Increase in R. S.
			S. P.	S. C.			
I Plant. Gran. and Dist. Water	Cont.	35.03	34.35	34.43	1.00	—	—
	Mould	36.88	− 8.40	1.4	30.00	33.03	29.0
	Yeast	35.39	33.9	34.65	1.0	—	—
	Combined Inoc.	35.42	34.0	33.79	1.0	.64	—
	Bact.	35.73	31.4	Gum	5.0	2.9	4.0
II Plant. Gran. and 1 cc per 100 of 1-1000 Mol. Sol.	Cont.	35.42	34.0	34.09	0.90	—	—
	Combined	35.37	32.55	32.74	1.90	1.35	1.07
	Yeast	35.25	33.65	33.44	1.17	0.65	0.27
	Bact.	40.30	29.15	—	5.71	4.85	4.81
III Plant. Gran. and 1 cc per 100 of 1-1000 Dirt Suspension	Combined	34.70	33.45	33.6	1.08	.3	0.28
	Cont.	34.62	33.95	33.97	0.8	—	—
	Yeast	34.62	33.65	33.36	0.95	.6	0.15
	Bact.	35.17	25.65	—	11.42	8.30	10.62
IV Plant. Gran. and .01% Sodium Phosphate	Yeast	35.25	35.25	34.46	—	—	—
	Combined	34.77	34.77	32.70	2.28	—	—
	Cont.	Lost	—	—	—	—	—
	Bact.	—	—	Gum	15.0	—	—

TABLE VII.---Continued

Experiment on the Deterioration of Sugar Solutions from B-Standard Granulated and Distilled Water.
Three weeks Incubation.

Series B	Inoculation	Corrected Brix	Sucrose		% R. S.	Loss in Sucrose	Increase in R. S.
			S. P.	S. C.			
I Stand. Gran. and Dist. Water	Cont.	34.17	26.35	27.65	9.09	—	—
	Mould	35.82	3.65	10.4	32.2	17.2	23.11
	Bact.	35.57	23.75	25.78	11.4	1.87	2.31
	Yeast	35.82	23.95	25.78	10.2	1.87	1.11
	Combined	35.82	23.55	25.78	12.5	1.87	3.41
II Stand. Gran. and 1cc1-1000 Mol. Sol.	Cont.	35.17	26.3	28.00	8.69	—	—
	Bact.	35.42	23.6	26.37	12.2	1.63	3.51
	Yeast	35.42	24.0	26.83	12.2	1.17	3.51
	Combined	35.42	23.5	26.2	11.7	1.8	3.02
III	Cont.	35.42	25.4	27.7	10.52	—	—
	Combined	34.85	24.3	27.07	10.81	0.63	0.3
	Yeast	35.14	25.3	27.7	10.25	—	—
	Bact.	35.14	27.0	29.02	8.0	—	—
IV Stand. Gran. and .01% Sodium Phosphate	Cont.	34.85	34.75	35.53	—	—	—
	Yeast	34.85	34.5	34.9	0.20	0.6	—
	Combined	34.85	34.7	35.25	0.40	—	—
	Bact.	34.85	32.9	33.84	2.05	1.69	1.8

From the above table it will be seen that the mould cultures caused a heavy inversion in both of the untreated sugar solutions. The destruction of sucrose was more rapid, of course, in the plantation granulated solution. The bacteria caused a greater deterioration throughout the entire Series A than in B. The addition of the foreign matter to the solution in Series A increased greatly the action of bacteria. The action upon the yeast was not reflected in the results. With the exception of the deterioration caused by the mould cultures, there was no very pronounced sucrose destruction in any of the B series. It appears, therefore, that the amount of impurities added was insufficient to equalize the difference in the susceptibility of the two solutions to deterioration. It would be an entirely false assumption, however, to conclude that the addition to sugars of such extraneous matter as that used in the preceding experiments would necessarily tend to hasten their deterioration. On the contrary, it is quite likely that just the reverse would be true. While the substances added to the solution acted solely as a source of nutrition for the microorganisms when they are added to a sugar, they may serve to naturally increase the density of the moisture film and thus decrease the amount of available moisture.

In the following table are given the results of a two-months' storage of a plantation granulated sugar to which the following substances were added:

A Control.
B Distilled water.
C Tap water.
D 1:1000 molasses solution.
E 1:1000 dirt suspension.
F .01% sodium phosphate.
G 0.1% sodium phosphate.
H .01% calcium chloride.
I 0.1% calcium chloride.
J .01% calcium sulphate.
K 0.1% calcium sulphate.

TABLE VIII.

Table Showing the Influence of the Addition of Various Substances upon the Deterioration of Plantation Granulated Sugar.
Two months Incubation.

Sample	% Moist.	Sucrose		% R. S.	Factor	Dry Basis	
		S. P.	S. C.			S. P.	S. C.
A...........	.07	99.15	99.12	—	.082	99.21	99.18
B...........	.47	96.65	96.72	1.47	.140	97.10	97.17
C...........	.47	97.15	96.73	1.38	.164	97.60	97.18
D...........	.27	97.40	97.77	1.19	.103	97.66	98.03
E...........	.27	98.20	98.23	1.11	.150	98.46	98.49
F...........	.37	97.40	97.92	1.51	.142	97.76	98.28
G...........	.27	97.90	97.66	1.11	.128	98.16	97.92
H...........	.45	97.45	97.74	1.38	.176	97.89	98.18
I...........	.32	97.90	98.00	1.14	.152	98.21	98.31
J...........	.27	97.60	97.39	1.38	.112	97.86	97.65
K...........	.45	97.90	98.31	1.25	.214	98.34	98.75

The results in the above table show that the greatest deterioration took place in the series in which only distilled water was used. Where an ingredient was added in more than one quantity the deterioration was always greatest where the smaller amount was used.

From the results of our experiments upon the comparative nutritive requirements of the three groups of micro-organisms in sugars, we may conclude that their rank with regard to the possibility of their having these requirements satisfied in most any type of sugar is as follows:

(1) Mould.
(2) Bacteria.
(3) Torula.

THE DEGREE OF ACIDITY OR ALKALINITY OF THE MOLASSES FILM.

We will next consider the extent to which the reaction of the solution may control the activities of the different groups of micro-organisms in sugar. We have already seen that the bacteria in sugars have a very low tolerance of either acid or alkali. To determine this tolerance of acid and alkali by the torula the following experiment was conducted: To a white sugar syrup was added varying quantities of N/1 H_2SO_4, and N/1 NaOH. The addition was made after the syrup had been sterilized and while it was still hot. The results are given in the following table:

TABLE IX.

Showing Results of Experiments upon the Influence of Reaction of Syrups upon the Action of Torula.
One month Incubation.
Ashland White Sugar Syrup.

Series	Corrected Brix	Sucrose		R. S.
		S. P.	S. C.	
A. .7 Acid Cult. I Yeast................	53.37	30.25	34.12	8.5
Cult. II Yeast..............	53.45	30.4	34.62	8.33
A. .7 Acid Control.....................	60.65	49.5	50.57	5.0
A. 1.7 Acid Culture I Yeast............	53.05	23.6	27.9	12.5
1.7 Acid Culture II Yeast............	53.38	24.3	28.86	13.3
1.7 Acid Control.....................	61.61	48.3	49.8	6.45
1.5 Alkaline Culture I..................	54.90	38.1	41.73	2.89
1.5 Alkaline Culture II.................	54.78	38.3	40.95	2.85
1.5 Alkaline Control.....................	60.16	49.9	50.7	5.00

From the above table it will be seen that the deterioration was much greater in the series having the highest acidity. It is interesting to note, however, that some inversion took place in the alkaline series. In the latter series we find a decrease in reducing sugar similar to that which takes place in very concentrated sugar solutions. It appears that in this series the inversion of sucrose was so retarded that the glucose was fermented more rapidly than it was formed.

If we try this same experiment, however, on a solution of higher density we will probably find that increasing the acidity does not extend the limit of density in which the torula can destroy sucrose.

TABLE X.

Part II D (3).
Experiment on the Influence of Acidity of Solution upon the Deterioration of Sugar Solutions Inoculated.
Culture I Torula.

Sample	Brix	Sucrose		R. S.	Acidity 10 cc req. N/10
		S. P.	S. C.		
A Control......	70.65	58.60	60.35	9.09	.09
A Inoc.........	66.30	62.95	62.84	1.02	5.82
B Control......	70.17	58.00	59.91	8.69	1.94
B Inoc.........	66.26	63.10	62.88	1.19	3.80
C Control......	69.39	56.60	55.70	9.76	1.94
C Inoc.........	66.06	62.00	61.63	1.19	2.90

A. + .1 cc N/1 H_2SO_4 – 200 cc syrup.
B. + .3 cc N/1 H_2SO_4 – 200 cc syrup.
C. + .6 cc N/1 H_2SO_4 – 200 cc syrup.

The above table gives the results of an experiment on the deterioration of a syrup of varying acidities by a torula culture. It will be noted that the action of the torula was practically the same in all cases and that no inversion took place. Although the acidity was twice as great in Series B as in Series A, the action of the torula was still confined to the destruction of glucose. These results seem to indicate that increasing the acidity of the molasses film does not appreciably extend the maximum limit of density at which the sucrose destroying power of the torula is completely suppressed.

In the following tables are given the results of a similar experiment upon the influence of the acidity of the solution upon the destruction of sucrose by moulds.

TABLE XI.

Influence of Titration upon the Deterioration of Syrups.
A--.95 Acid.
Incubation one month.

Series A	Culture No.	Corrected Brix	Sucrose		% R. S.	Acidity 10 gm cc N/10 Req.	Loss in Sucrose	Increase in R. S.
			S. P.	S. C.				
A........	Cont............	62.40	59.2	59.45	1.19	1.0	—	—
0.95 Acid.....	Mould I.........	62.08	25.6	32.42	9.43	6.2	27.03	8.24
"	" II.........	62.61	22.9	30.46	10.20	3.5	28.99	9.01
"	" VI.........	62.03	57.7	58.05	2.71	1.0	1.40	1.52
"	" VII......	62.28	23.9	32.22	7.46	4.0	27.23	6.27
"	" VIII.....	62.53	23.8	32.14	5.61	3.4	27.31	4.42
"	" XI........	62.48	39.4	43.56	18.51	3.7	15.89	17.32
"	" XII......	62.31	41.9	45.37	17.85	2.4	14.08	16.66
"	" XIII.....	62.33	25.5	32.48	Lost	4.7	26.97	—
"	" XIV......	62.03	35.0	39.63	Lost	1.0	19.72	—
"	Yeast I..........	60.31	56.5	56.69	Lost	3.5	2.76	—

TABLE XII.

Influence of Titration upon the Deterioration of Syrups.
B- .55 Alk.
Incubation, one month.

Series B	Culture No.	Corrected Brix	Sucrose		% R. S.	Acidity 10 gm. cc N/15 Req.
			S. P.	S. C.		
.55 Alk......	Mould I.................	61.89	22.9	24.37	23.80	6.9
"	" II................	62.29	26.1	33.44	26.66	3.7
"	" VI................	61.74	57.2	57.80	2.64	1.0
"	" VII..............	62.04	24.4	31.95	24.39	3.9
"	" VIII..............	62.38	21.0	22.87	26.31	4.7
"	" XI................	61.92	51.3	53.15	3.49	1.0
"	" XII..............	61.87	58.1	58.64	2.33	0.8
"	" XIII..............	62.12	28.1	34.77	21.73	5.1
"	" XIV..............	62.67	3.7	9.10	20.00	0.9
"	Yeast I.................	59.07	55.3	55.97	0.64	3.7

A comparison of the results given in the two above tables shows that the inverting action of the mould cultures upon the sucrose in the different solutions was very pronounced in both of the series. In some cases a culture would cause a much higher inversion in Series A than in B, and with other cultures the reverse would be true. For example, observe the greater deterioration induced by Culture XIV in Series B than in Series A. Also the greater deterioration induced by Culture XII in Series A than in Series B. On the whole, therefore, it appears that, considering the group of mould cultures as a whole, they produced approximately the same amount of deterioration in both cases. This indicates that they can adapt themselves to a rather wide range of reaction. The torula culture used in the experiment induced some slight deterioration in both cases. In Series B the activities of the torula resulted in greatly decreasing the amount of glucose originally present in the solution. Unfortunately, the control flask in the series was lost, and also the flask inoculated with torula in the other series. So that we cannot compare the relative degrees of glucose destruction in the two cases.

It will be noted that the increase in acidity in the solution inoculated with torula is about equal to that resulting from the mould growth. It seems likely that it is due to this formation of acid that the torula is able to invert sucrose in an alkaline solution. The inversion probably does not begin until the fermentation of the glucose already present in the solution yielded sufficient acid to correct the alkalinity of the solution.

We may well assume from these experiments that moulds are not easily rendered inactive toward sucrose by slight changes in the reaction of the solution.

PART III.

THE INFLUENCE OF THE DENSITY OF THE MOLASSES FILM UPON THE DETERIORATION OF SUGARS.

In a previous part of this publication we advanced the theory that the validity of the Factor of Safety $\frac{W}{100\text{-}P} = .333$ depends upon the fact that it is an expression of a definite degree of density of the molasses film surrounding the sugar crystal. We will now subject this theory to a more critical study.

If we take any sugar and divide it up into several samples and allow these samples to absorb varying amounts of moisture, we should expect the rate of deterioration to vary with the moisture content. That is to say, we should expect the rate of deterioration to increase as the ratio of solid non sucrose to moisture decreased. The following table shows the results of an experiment, the purpose of which was to test this supposition. A Cuban 96-test sugar was taken and thoroughly mixed into eight equal portions. Samples A (1), (2) and (4) were allowed to lose some of their original moisture, while the remaining samples, with the exception of No. 3, were placed under moist chambers and allowed to take up moisture. A series of samples was then obtained, having a range of solid non sucrose moisture ratios from .207 to .747.

TABLE I.

Experiment on the Influence of Factor of Safety upon Deterioration of 96-Test Sugar. Incubation one month.

Sample	Original Factor	% Moist.	Sucrose		% R. S.	Dry Basis		Factor
			S. P.	S. C.		S. P.	S. C.	
A (1)....	.207	0.41	97.50	98.47	.543	97.90	98.87	.164
A (2)....	.220	0.57	97.25	98.09	.444	97.80	98.65	.207
A (4)....	.274	0.62	97.20	98.05	.520	97.80	98.66	.221
A (3)....	.392	0.90	97.00	97.28	.480	97.88	98.16	.300
A (6)....	.674	3.95	87.60	89.40	4.20	91.20	93.07	.318
A (5)....	.699	4.14	87.70	89.13	2.66	91.48	92.97	.336
A (7)....	.743	4.05	88.00	89.45	4.87	91.71	93.22	.337
A (8)....	.747	4.26	86.15	87.98	5.88	89.97	91.86	.307

ABOVE AFTER SIX MONTHS

A (1)....	.207	0.52	97.5	97.92	.555	98.00	98.42	.115
A (2)....	.220	0.61	96.8	97.12	.537	97.39	97.71	.190

A glance at the above table shows that no appreciable deterioration took place in any of the samples having a factor of less than .674. The increase in the moisture contents of A (6) over A (3), the next lower in the series, was more radical than was desired, since it would have been better to have had several samples intervening between the two. However, this does not obscure the real purpose of the experiment, which is to interpret the results in the light of the probable density of the molasses film in each case. In A (3) we have the original sample with its normal moisture content. Let us assume that the molasses on this sugar is of 75 Brix density, which is more likely an overestimate than an underestimate of its density. Sample A (6) has absorbed over three times its original amount of moisture, so the density of the film is probably not more than one-half its original density. It would probably be incorrect to assume that the molasses films of the crystal could be diluted to the full extent of the moisture absorbed without it taking some of the sucrose crystal into solution. Nevertheless, the film must be considerably diluted as a result of moisture absorption, for otherwise the increase in moisture would not affect the deterioration of the sugar. We must assume, therefore, that the molasses film surrounding a sugar crystal can exist in a more dilute state in the form of a film than when an equal amount of water is added directly to sugar. In other words, the crystal seems to offer a certain resistance to the solvent action of the film moisture. If we examine the table showing the analyses of samples A (1) and (2) after six months' storage we will find that no deterioration took place. It appears that in those samples the molasses films were of too great a density for the development of microorganisms.

For the purpose of determining the influence of the density of the molasses film upon sugar deterioration it is preferable to use sugars with films of known composition. It thus becomes necessary to make sugars from magmas, in which the ratio of sugar crystals to molasses is known. With this idea in view, a series of sugars were made in the laboratory. A standard granulated sugar furnished the crystals, and a series of syrups were made from varying mixtures of a white sugar syrup and a

Louisiana blackstrap molasses. The analyses of the original syrup, the finished sugars and the resulting molasses are given in the following tables, respectively:

TABLE II.

Experiment on the Deterioration of Laboratory made Sugar and Molasses.

ORIGINAL ANALYSIS OF SUGAR

Sample	Proportion of Mixture	% Moist.	Sucrose		R. S.	Dry Basis		Factor
			S. P.	S. C.		S. P.	S. C.	
I	White from 99 purity	.61	99.1	99.24	—	99.7	99.84	.677
III	⅔ White Sugar ⅓ B. S.	.85	98.2	98.6	7.25	99.04	99.44	.472
IV	½ W. S. ½ B. S.	.85	97.7	98.4	0.43	98.53	99.23	.369
V	⅓ W. S. ⅔ B. S.	1.04	97.00	97.26	0.80	98.01	98.27	.346

TABLE III.

ORIGINAL ANALYSIS OF MOLASSES

Sample	Proportion of Mixture	Brix	Sucrose		R. S.	Purity	Factor
			S. P.	S. C.			
I	White from 99 purity	69.16	68.45	68.25	—	98.9	.977
III	⅔ W. S. ⅓ B. S.	72.17	52.00	54.31	8.00	72.05	.579
IV	½ W. S. ½ B. S.	73.82	47.20	49.50	9.5	63.90	.495
V	⅓ W. S. ⅔ B. S.	74.55	40.00	43.80	12.9	53.6	.440

TABLE IV.

ANALYSIS OF SYRUPS USED IN MAKING SUGAR

Sample	Proportion of Mixture	Brix	Polarization	Purity	Factor
I	Pure White solution	67.64	67.00	99.05	.977
III	2 prts. sug. sol., 1 pt. B. S.	71.43	53.20	74.47	.610
IV	1 part I, 1 part B. S.	73.54	48.00	65.27	.508
V	1 part I, 2 parts B. S.	74.78	44.00	58.80	.450

A comparison of Tables III and IV shows that the Brix of the molasses is slightly higher in certain cases than that of the corresponding syrups. This was due to the unavoidable passage of some fine grains of sugar through the centrifugal screen.

In the following table are given the results of the analyses of sugars and molasses after an eight-month storage period:

TABLE V.

Experiment on Deterioration of Laboratory made Sugar and Molasses.---Continued.

ANALYSIS OF SUGAR AFTER EIGHT MONTHS

Sample	Proportion of Mixture	Sucrose		% Moist.	Dry Basis		R. S.	Loss in S. C.	Increase in R. S.	Factor
		S. P.	S. C.		S. P.	S. C.				
I	White from 99 purity.......	98.5	98.68	0.30	98.79	98.97	.510	—	—	.200
III	⅔ W. S. ⅓ B. S..........	96.65	96.85	0.85	97.48	97.68	1.58	1.29	1.07	.253
IV	½ W. S. ½ B. S..........	96.0	97.26	0.90	96.87	98.14	1.14	0.83	0.63	.225
V	⅓ W. S. ⅔ B. S..........	96.6	96.96	1.10	97.67	98.03	1.06	0.94	0.55	.323

The results given in the above table show that the rate of deterioration of the different sugars is, with one exception, what was to have been expected from their respective "factors of safety." The exception is with the white sugar, which might have been expected to deteriorate. In that case it is quite likely that although the ratio of solids non sucrose to moisture was low, the actual moisture present was insufficient for the development of micro-organisms. Regarding this condition we will have more to say in a subsequent portion of this publication.

TABLE VI.

ANALYSIS OF MOLASSES AFTER EIGHT MONTHS

Sample	Proportion of Mixture	Brix	Sucrose		R. S.	Loss in S. C.	Increase in R. S.
			S. P.	S. C.			
I	White from 99 purity..............	69.64	50.7	54.39	13.3	10.37	8.76
III	⅔ W. S. ⅓ B. S.................	70.49	43.2	46.18	14.9	7.24	5.34
IV	½ W. S. ½ B. S.................	71.09	37.6	42.31	16.12	4.08	5.14
V	⅓ W. S. ⅔ B. S.................	71.38	27.60	35.07	20.8	2.67	5.5

In the above table are given the results of the analyses of the molasses from the sugars after a similar period of storage. It will be observed that the rate of deterioration of the molasses samples is in inverse proportion to their density, and increases directly with the decreasing ratio of solids non sucrose to moisture.

The deterioration of these molasses samples with densities of 73 and 74 Brix does not find an exact parallel in the results of any of our experiments upon the inoculation of syrups and molasses. However, it is very probable that sufficient moisture was absorbed and held as a surface film to form a zone of suitable density for the development of micro-organisms. The molasses samples were kept in jars, which were provided with metal covers, but these did not form an entirely airtight cover. This seems all the more likely when we consider that the storage period was eight months. While the moisture content of the sugars did not vary appreciably during the storage period, yet it was possible that they might have absorbed some moisture during the period intervening between the time that they were made and the time of the analysis.

In the next experiment it was decided to avoid all possibility of the sugars absorbing moisture from the air, in order to determine whether this moisture is essential to the deterioration of sugars in which the ratio of solids non sucrose is less than three to one. A question that has often been raised in connection with the subject of sugar deterioration is whether or not the molasses films of sugars are homogeneous. If sugar deterioration depends, as some would argue, upon the fact that the outer periphery of the molasses film is much more dilute than the interior, then the deterioration of two sugars with the same factor might be different in case one had absorbed moisture and the other had not. We should, therefore, be able to demonstrate the truth of this theory by making sugars with films of known composition and storing them in sealed containers, and comparing them with sugars which have attained the same factor by the absorption of moisture. An experiment, therefore, was conducted as follows: A coarse standard granulated sugar was used for the experiment. The series of sugars were made with the use of the following syrups:

A. Final molasses alone.
B. ⅚ final molasses + ⅙ white sugar syrup.
C. ⅔ final molasses + ⅓ white sugar syrup.
D. ½ final molasses + ½ white sugar syrup.

The following table gives the results of the analyses of the resulting molasses:

Series	Corrected Brix	R. S.	Sucrose	Reducing Sugar
			Clerget	
A	75.3	29.6	37.42	19.2
B	74.29	36.30	41.25	16.4
C	72.39	41.0	44.69	14.2
D	70.14	46.50	49.88	9.6

After drying the sugars in a laboratory centrifugal each sample was thoroughly mixed and divided into several portions. One portion was immediately placed in a glass jar with ground glass stopper and sealed with paraffin. Another portion was analyzed, and the remaining portion placed in moist chambers and allowed to absorb moisture. Samples A, B, C and D are the original samples kept in sealed containers. Samples A. I, A. I (1), B. I, C. I and D. I are the samples that absorbed moisture. The following table gives the per cent of molasses that the different sugars retained, as calculated from their respective moisture contents; also the per cent of reducing sugar found by analysis, the amount calculated from the calculated molasses, and also the calculated sucrose in the film:

Sample	Calculated % Molasses	R. S. Found	Calculated R. S.	Calculated Sucrose
A	4.44	.98	.84	1.66
B	3.38	.55	.69	1.39
C	2.89	.41	.51	1.29
D	4.34	.411	.52	2.16

TABLE VII.

Analysis of Sugars made in Laboratory.
Incubation three months.

Sample	Factor	% Moist.	SUCROSE Dry Basis S. P.	SUCROSE Dry Basis S. C.	% R. S.	Loss in S. C.	Increase in R. S.
A—original	.314	1.1	97.57	97.73	.98	—	—
A—3 mo. Storage	.400	1.2	98.17	98.22	1.04	—	.06
AI—original	.383	1.48	97.57	97.73	.98	—	—
AI—3 mo. Storage	.361	1.32	97.63	97.62	1.20	.11	.22
AI (1) original	.395	1.55	97.57	97.73	.98	—	—
AI (1) 3 mo. Storage	.333	1.32	97.28	97.19	1.18	.54	.20
B—original	.328	.87	98.21	98.62	.69	—	—
B—3 mo. Storage	.280	.85	97.83	98.22	.95	.40	.26
BI—original	.362	1.27	98.21	98.62	.69	—	—
BI—3 mo. Storage	.340	1.02	97.99	97.85	1.04	.77	.35
C—original	.333	.80	98.38	98.55	.51	—	—
C—3 mo. Storage	.275	.80	97.88	98.14	.98	.41	.47
CI—original	.485	1.49	98.38	98.55	.51	—	—
CI—3 mo. Storage	.350	1.40	97.36	97.51	1.35	1.04	.84
D—original	.464	1.30	98.48	98.59	.52	—	—
D—3 mo. Storage	.367	1.27	97.79	96.72	1.16	1.87	.64
DI—original	.544	1.76	98.48	98.59	.52	—	—
DI—3 mo. Storage	.353	1.52	97.06	97.28	1.38	1.31	.86

A—Made from coarse granulated sugar + Final molasses.
B—Made from coarse granulated sugar + 5/6 Final molasses, 1/6 White S. Syrup.
C—Made from coarse granulated sugar + ⅔ Final molasses, ⅓ White S. Syrup.
D—Made from coarse granulated sugar + ½ Final molasses, ½ White S. Syrup.

The results given in the above table show that of the samples of the sugars which were prevented from absorbing moisture only one deteriorated appreciably in storage, and that was "D" with a factor of .464. Series C, with a factor of .333, held its polarization. If we take C. I and D for comparison we have two sugars with approximately the same factors, but with the difference that the former has absorbed .6 per cent of moisture and the latter none. If absorbed moisture creates especially favorable conditions for deterioration, then the deterioration in C. I should be greater than in D. The results show no such difference. In fact, the deterioration in the two cases is as nearly equal as we could expect from the close agreement of the solid non sucrose moisture ratios. While the other samples do not agree as closely in their factors and therefore are not as favorable for comparison, yet the results in no single instance indicate that any especially favorable condition for sugar deterioration is created by moisture absorption beyond what is indicated by the solid non sucrose moisture ratio. If we refer to the density of the molasses from this sugar we find that molasses D had a Brix of 70.14. The next in the series had a density of 72.39. Of the original sugars, only D deteriorated, which rather tends to confirm our experiments on the limit of density for sucrose destruction by the group of micro-organisms in sugars.

That the rate of deterioration of a sugar is to a large extent comparable to the deterioration of a molasses or syrup of a composition similar to the molasses film of such a sugar is shown by the following experiment. In this experiment one of the plantation white sugar syrups, No. A, used in Part II, was employed. A coarse standard granulated sugar was used in making up the magma. The sugar was dried in the laboratory centrifugal as in the previous experiments. The syrup used was divided into two portions, one of which was neutralized with lime, the other allowed to retain its original acidity, which was such that 1.3 cc of N/10 NaoH was required for each 10 grams.

TABLE VIII.

Analysis of Laboratory Sugars Sterlized in Autoclative 15′ at 15 lbs. pressure Reserve White Sugar Syrups used @ 63 Brix.

Sample	Treatment	% Moist.	Sucrose		R. S.	Dry Basis		Factor
			S. P.	S. C.		S. P.	S. C.	
4	Acid Sugar Control	.85	98.30	98.49	.27	99.14	99.33	.500
2	Acid Sugar Inoc. Bact.	.42	97.50	98.04	.66	97.91	98.45	.168
3	Acid Sugar Inoc. Mould	.97	96.50	97.12	1.60	97.44	98.06	.277
1	Acid Sugar Inoc. Yeast	1.05	98.35	98.61	.2	99.39	99.65	.636
7	Neutral Control	.92	98.45	98.69	.5	99.36	99.60	.593
6	Neutral Inoc. Bact.	.35	96.30	96.92	1.90	96.44	97.06	.094
8	Neutral Inoc. Mould	.87	96.80	97.38	1.30	97.64	98.22	.271
5	Neutral Inoc. Yeast	.70	98.70	98.99	.31	99.39	99.68	.538
11	Neutral Abs. Moist Control	1.15	98.15	98.26	.50	99.29	99.40	.621
10	Neutral Abs. Moist Inoc. Bact.	1.50	97.40	97.50	.6	98.88	98.98	.576
12	Neutral Abs. Moist Inoc. Mould	1.20	94.76	95.23	2.33	95.91	96.38	.229
9	Neutral Abs. Moist Inoc. Yeast	1.02	98.00	98.15	.41	99.00	99.15	.510
13	Acid Sugar Abs. Moist Inoc. Bact.	1.02	98.15	98.42	.28	99.16	99.43	.551

A study of the above table shows that with the exception of Sample No. 6, only the samples inoculated with moulds deteriorated. The deterioration caused by mould was greater in the acid than in the neutral sugar, but greatest of all where the neutral sugar was allowed to absorb moisture, in which case it acquired a factor of .621. It appears strange at first sight that the bacteria should have caused a deterioration of this sugar, when the same culture caused no deterioration in the original syrup. (See Table III, Part II-D.)

A microscopical examination of this sugar sample, together with plates made from it, showed that the apparent inconsistency in results was due to a contamination with mould. Both the microscopical examination of the sugar and the plates made from it showed a predominance of mould in the sample. Neither the bacteria nor the yeast cultures seemed able to cause any appreciable deterioration of the sugars. To determine the action of the bacteria upon a sugar with a lower ratio of solids non sucrose to moisture, the following experiment was conducted: A white sugar magma was made in the laboratory and dried as in the previous experiment. The syrup used in making this magma was divided into two portions, one of which was neutralized with lime and the other acidified with SO_2. The Brix of this syrup was 65.7.

TABLE IX.

Table Showing Results of Inoculation of Laboratory Made Sugar with Culture of Bacteria.
Incubation period one month.
A. White Sugar.

Series	Reaction	Treatment	Date	S. P.	S. C.	Moist.	Ratio Solids Non Suc. to Moist.	Dry Basis		Moist.	Original Analysis
								S. P.	S. C.		Ratio Solid Sucrose to Moist.
I	Neut.	Cont.	6/21	97.8	98.07	1.22	.554	99.00	99.28	1.57	.747
		Inoc.	"	97.55	97.72	1.55	.632	99.08	99.25	1.40	.721
	Acid	Cont.	"	97.9	98.30	1.19	.566	99.07	99.48	.92	.557
		Inoc.	"	98.0	98.22	0.92	.460	98.90	99.13	1.17	.624
II	Neut.	Cont.	"	98.3	98.23	0.71	.417	99.00	98.93	.42	.428
		Inoc.	"	97.8	98.07	1.05	.477	98.82	99.11	.60	.660
	Acid	Cont.	"	98.3	98.46	0.82	.482	99.11	99.27	.55	.426
		Inoc.	"	97.9	97.99	1.08	.514	98.96	99.05	1.10	.604
III	Neut.	Cont.	"	97.4	97.75	1.91	.734	99.29	99.65	1.23	.694
		Inoc.	"	97.6	97.91	1.80	.750	99.38	99.70	1.57	.744
	Acid	Cont.	"	96.8	96.81	2.11	.659	98.88	98.89	1.55	.682
		Inoc.	"	97.8	97.90	1.63	.740	99.42	99.52	1.35	.648

ANALYSIS OF ORIGINAL SUGAR

Series	Sucrose		Moist.	Ratio Solids Non Suc. to Moist.	Dry Basis		Molasses	
	S. P.	S. C.			S. P.	S. C.	Titre	Brix
Acid	98.5	98.6	0.77	.513	99.26	99.36	1.1 cc	65.76
Neutral	98.6	98.62	0.85	.614	99.44	99.46	Neut.	65.76

The results in the above table show that no appreciable deterioration took place in any of the inoculated samples, in spite of the fact that the solid non sucrose moisture ratio was considerably under the point of safety. However, this was what should have been expected in consideration of the density of the syrup used in making the sugars and which constituted their films. A similar experiment was next tried on a laboratory-made 96-test sugar. The molasses used in making the magma was, of course, much higher in density than that used in the previous experiment. The reaction of the molasses was adjusted as in the previous experiment. The sugars were divided into three lots and allowed to absorb different amounts of moisture. In some instances the moisture content of the sugar was over two per cent.

TABLE X.

Table Showing Results of Inoculation of Laboratory Made Sugars with Culture of Bacteria.
B. 96-Test Sugar.

Series	Reaction	Treatment	Date	S. P.	S. C.	Moist.	Ratio Solids Non Suc. to Moist.	Dry Basis		Moist.	Original Analysis
								S. P.	S. C.		Ratio Solids Non Sucrose to Moist.
I	Neut.	Cont.	6/23	97.3	97.27	1.37	.507	98.65	98.62	1.65	.563
		Inoc.	"	97.4	97.67	2.14	.823	99.52	99.80	1.97	.609
	Acid	Cont.	"	96.8	96.88	1.32	.412	98.09	98.17	1.65	.563
		Inoc.	"	97.2	97.20	1.26	.450	98.44	98.44	1.47	.546
II	Neut.	Cont.	"	96.2	96.25	1.93	.507	98.09	98.14	1.87	.595
		Inoc.	"	96.4	96.84	1.49	.413	97.85	98.30	1.85	.612
	Acid	Cont.	"	97.1	97.20	1.06	.365	98.14	98.24	1.22	.409
		Inoc.	"	97.3	97.27	0.90	.333	98.18	98.15	1.12	.476
III	Neut.	Cont.	"	96.5	96.92	1.66	.474	98.12	98.55	1.92	.603
		Inoc.	"	96.3	96.45	2.06	.556	98.32	98.47	2.50	.670
	Acid	Cont.	"	96.5	96.64	1.91	.545	98.37	98.53	1.90	.612
		Inoc.	"	96.8	97.15	1.52	.475	98.29	98.64	2.00	.626

ANALYSIS OF ORIGINAL SUGAR.

Series	Sucrose		Moist.	Ratio Solids Non Suc. to Moist.	Dry Basis		Molasses	
	S. P.	S. C.			S. P.	S. C.	Titre	Brix
Acid	98.2	98.28	0.56	.311	98.75	98.83	10 cc	74.49
Neutral	98.0	98.35	0.69	.345	98.68	99.03	0.4 cc	74.49

The results of the above table show that no deterioration took place in any of the inoculated samples. The conditions in both acid and neutral samples were alike unfavorable for the action of the bacteria. Evidently the molasses was of too high a density to allow the bacterial development to take place.

We have already referred, in a previous section of this report, to the characteristic manner in which torula destroy the reducing sugar in raw sugars and thereby increase their polarization. The following table gives an example of this selective action of the torula cultures upon reducing sugars:

TABLE XI.

Experiment on Deterioration of Sugars Inoculated with Torula Cultures.

Inoculation	Sucrose		Reducing Sugars	Moist.	Period of Inoculation days	Moisture Free Basis		Decrease in S. C.	Increase in R. S.	Factor
	S. P.	S. C.				S. P.	S. C.			
No. 7 96 Control	92.6	92.60	1.42	4.08	30	96.56	96.56	—	—	.550
Cult. I Yeast	94.0	93.71	0.41	4.36	"	98.23	97.98	+ 1.42	− 1.01	
Cult. II	93.6	93.96	0.392	4.33	"	97.84	98.21	+ 1.65	− 1.03	
Cult. III	93.5	93.91	0.40	4.75	"	98.16	98.59	+ 2.03	− 1.02	
No. 8 Control	90.1	91.19	3.88	5.12	"	95.00	96.20	—	—	—
No. 2 Cult. I	92.0	91.97	2.67	5.39	"	97.30	97.30	+ 1.10	− 1.21	—
Cult. II	91.8	92.02	2.75	5.58	"	97.30	97.50	+ 1.30	− 1.13	—
Cult. III	91.3	91.54	2.98	5.23	"	96.40	96.70	+ 0.50	− .90	—

In the above table it will be noted that in almost every case the inoculation of the sugar with torula resulted in a marked decrease in the per cent of reducing sugars remaining in the sample at the end of the experiment.

EXPERIMENT No. XII.

B. III.

Sugars Inoculated. Absorbed Moisture.

Series	% Moist.	Sucrose		Dry Basis		Original Factor	Final Factor	% R. S.	Inoculation
		S. P.	S. C.	S. P.	S. C.				
III									
B. III (4)....	2.79	95.1	95.76	97.82	98.48	.699	.569	.691	Yeast
B. III (1)....	1.67	96.4	97.08	98.03	98.71	.723	.463	.684	Bact.
B. III (2)....	2.72	94.1	94.68	96.73	97.31	.682	.444	1.412	Mould

The above table gives the results from an experiment on the inoculation of the same sugars as used in the previous experiment with a culture of bacteria, yeast and mould, respectively. Only the sample inoculated with mould deteriorated during the incubation period of one month. It will be noted that the ratio of solid non sucrose to moisture was low in all cases. The solid non sucrose moisture ratio was least favorable to deterioration in the sample inoculated with mould.

PART IV.

PRACTICAL APPLICATION OF THE RESULTS OF THE INVESTIGATION TO THE CONDITIONS EXISTING IN THE CANE SUGAR INDUSTRY.

We will now endeavor to ascertain the extent to which the principles established by our investigation are susceptible of direct application to industrial conditions as a preventive measure against losses from sugar deterioration. The measures for the prevention of sugar deterioration may, for convenience, be classed under three distinct heads:

(1) The manufacture of sugars which conform in composition to the best standards of keeping quality.
(2) Taking precautionary measures to prevent the excessive infection of sugars with deteriorative micro-organisms.
(3) Providing suitable storage conditions for sugar.

When we consider the composition of sugar with special reference to its influence upon their deterioration, we are irresistibly drawn to the conclusion that the most important factor in this particular connection is the density of the molasses film surrounding the crystal. It is upon this fact that the validity of the solid non sucrose moisture ratio seems to depend. Since the density of the film is the all-important factor in sugar deterioration, it is of importance to find out how this principle can be utilized in factory practice. Here we have, for example, the practice of washing sugars in the centrifugal up to higher polarization tests. This practice results necessarily in diluting the molasses film and therefore lowers the solid non sucrose moisture ratio. Therefore, a washed sugar deteriorates with a much lower moisture content than the same sugar would otherwise have done. The validity of the ''Factor of Safety'' establishes the principle that the safety of a sugar from deterioration is entirely independent of the amount of molasses it contains. In other words, it is the composition of the molasses film, and not the crystal that it surrounds, which determines the susceptibility of a sugar to deterioration. However, it is of course obvious that the extent to which a sugar can lose in polarization from deterioration depends on the amount of molasses surrounding the crystal. This fact is very clearly shown in the following table, which was prepared by Mr. F. B. Brenneman, superintendent of the Rio Cauto factory, who has kindly authorized me to use it. The explanation of the table and the discussion of it is quoted directly from an article submitted to the writer by the above-named author.

TABLE I.

Polarization	85 Brix 38.25 Sucrose 45 Purity			83.75 Brix 41.88 Sucrose Molasses 50 Purity			82.5 Brix 45.375 Sucrose 55			81.25 Brix 48.75 Sucrose 60			80 Brix 52 % Sucrose 65		
	% Mol.	Moisture	Factor	% Mol.	Moisture	Factor	% Mol.	Moisture	Factor	% Mol.	Moisture	Factor	% Mol.	Moisture	Factor
94.00	9.71	1.46	.243	10.32	1.68	.280	10.98	1.92	.320	11.71	2.20	.366	12.49	2.50	.417
94.5	8.91	1.34	.243	9.46	1.54	.280	10.07	1.76	.320	10.73	2.00	.364	11.45	2.29	.417
95.00	8.10	1.215	.243	8.60	1.40	.280	9.15	1.60	.320	9.76	1.82	.364	10.41	2.08	.416
95.50	7.29	1.094	.243	7.74	1.26	.280	8.23	1.44	.320	8.78	1.64	.364	9.37	1.87	.416
96.00	6.48	.972	.243	6.88	1.12	.280	7.32	1.28	.320	7.80	1.46	.365	8.33	1.66	.415
96.50	5.67	.85	.243	6.02	.98	.280	6.41	1.12	.320	6.83	1.28	.366	7.29	1.46	.417
97.00	4.86	.729	.243	5.16	.84	.280	5.492	.96	.320	5.85	1.10	.366	6.25	1.25	.417
97.5													5.20	1.04	.416
98.0													4.16	.83	.415

This Table Calculated — Assuming sugar grains 100 % Sucrose.

The resulting sugars being pure grains coated with molasses of varying purities: Then a sugar Pol. 94 −

$$\begin{array}{rlcl} 100. & & & \\ 9.71 & \%\ \text{Mol.} \times 38.25 & = & 3.714 \\ \cline{1-1} 90.29 & \text{grains} & = & 90.29 \\ \cline{4-4} & & & 94.004\ \text{P. of sug.} \end{array}$$

The Brix, and Sucrose to correspond to Molasses of varying purities assumed.
No water used in centrifugals.

A very interesting and important practical point is mentioned in the discussion of the preceding table by the author. The point in question is the relative keeping qualities of two sugars with the same "factor of safety," but with different polarization. The margin of safety is greater, as Mr. Brenneman points out, in the sugar with the lower polarization, since it can absorb more moisture without attaining a dangerously low ratio of solids non sucrose to moisture.

According to the formula given by Deerr & Brown used in Australia, the sugar may be considered safe if

$$\frac{\text{Moisture \%}}{100 - \text{Pol.}} = 33\ 1/3.$$

If we consider that grains of sugar are practically pure, then if sugar was dried in centrifugals without washing, the moisture and the factor 100 — Pol would be the moisture and non-sucrose of the molasses; therefore, the factor of safety would remain the same regardless of per cent of molasses left in sugar, since the moisture and non-sucrose would increase or decrease in same proportion.

Reasoning along this line, sugar made which yields a high purity molasses would not have as good keeping quality as sugar yielding lower purity molasses, since with the higher purity molasses surrounding crystals the moisture would be in larger proportion to the non-sucrose, where polarization of sugar is the same.

The following table shows this approximately:

"In the preceding table it is shown that in centrifugalling without washing, that sugar made giving off a molasses of a certain purity, will have same factor of safety regardless of polarization or degree of completeness which molasses is removed."

"That the factor of safety is better with sugars giving low purity molasses than where high purity molasses is given off.

That a 96 test sugar from high purity massecuite will be required to retain a larger per cent of molasses and a larger per cent of moisture than where lower purity is used, and factor of safety not as good."

"In working in tropics where we have high purity syrups we find two methods of pan work used:"

System I—Making first massecuite nearly pure syrup—say, 80-85 purity. Mol. 60-65. Mixed massecuite 72-75 purity. Mol. 45-50. Low grade massecuite 60 purity. Mol. exhausted.

System II—First massecuite 75-78 purity. Mol. 54-55. Second massecuite 60 purity. Mol. exhausted.

"For a number of reasons System No. 1 is preferred:

Molasses given absolute change: Possible thus to get low purity mixed molasses to take back on the third grade M. C.

As to sugar made, that from high purity M. C. will have high polarization and low moisture if grain is large and centrifugal speed good. But as shown in table, factor is bad."

"That from low purity M. C. will have lower polarization, though if polarization of the other sugar is high enough this sugar may be dried without water and mixed with higher sugar giving desired polarization. In this case, we have a mixture of one sugar with low factor and one with high factor, but as these are mixed only mechanically and molasses on two sugars being separate, we cannot consider the average analysis as representing the factor of sugar which gave off highest molasses, as according to the theory, this sugar should be first to deteriorate.

"It would thus appear that for making good storage sugar, if we can depend on formula $\frac{H_2O}{100-P}$*, that the best sugar would result where we use the two massecuite system in case sugar not over 96 is desired. By running purity of first massecuites as low as possible to get 96 test without washing we would then expect to get sugar with highest possible factor as shown by table.*

"If we use three massecuite system, we may get lower average moisture and high average polarized sugar, but according to formula keeping quality not as good."

"Also with the low moisture sugars and at same time low factor sugars, it will take less absorption of moisture to change factor to unsafe. For example we have a sugar 97 polarization, .96%

moisture with a factor .320, this being less than .333 we would call it safe. Suppose it now absorbs .3% moisture. It would then have pol. 96.71, moisture 1.26, giving factor .383, which would be considered unsafe."

Take now a sugar 96 pol. 1.12 moisture. Let this absorb .3% moisture; we have pol. 95.71, moisture 1.42, factor .331 which would still be safe."

"Now another consideration, which sugar will be the most hygroscopic? Will the lower polarization sugar absorb more moisture than the sugar of higher polarization? If such is true, the sugar having the better factor of safety might absorb additional water and become unsafe sooner than the sugar which gave off high purity mol., and has only a narrow margin of safety."

Evidently one of the surest means of manufacturing raw sugars which will not deteriorate in storage is to keep the density of the molasses films surrounding their crystals beyond 75 Brix. This applies, of course, only to raw sugars, as the keeping quality of white sugar must, in the nature of the case, depend upon their absolute dryness. But to manufacture sugars with a polarization of 96, with films of very low purity molasses, necessitates a thorough purging of the crystals so that they will retain only a small amount of their molasses; otherwise the polarization of the sugar would be too low. However, a good purging can only result from good and effective clarification, and it is to the deficiency in this respect that much of the trouble from deterioration is due. If the massecuite is viscous from excess of lime it does not purge well, and excessive amounts of water have to be used in the centrifugal. In regard to the theory of the relation between film density and sugar deterioration, Browne[1] points out that a case once came under his observation where a sugar house superintendent made it a practice to wash sugars in the centrifugal with low grade molasses instead of water. Although this practice resulted in the entire prevention of deterioration in storage, it is improbable that the superintendent fully understood the scientific principle upon which its success depended.

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[1] Chemical Factor in the Deterioration of Raw Cane Sugars.

In connection with the consideration of the composition of sugars in its relation to deterioration, we should mention the influence of size of grain.

THE INFLUENCE OF SIZE AND HARDNESS OF GRAIN UPON SUGAR DETERIORATION.

It is an obvious fact that the smaller the grain of a sugar the greater will be the amount of film surface exposure. Hence, other conditions being equal, a small grain sugar is more apt to deteriorate than one with a larger grain, because it is harder to purge properly and offers more surface for moisture absorption.

Regarding the influence of hardness of the grain upon sugar deterioration but little seems to be known. It is generally believed, however, that the hardness of the grain plays a considerable part in protecting sugars against such changes. Hard grain sugar appears less hygroscopic than sugars of the soft grain type. However, the impression may have been obtained simply from the fact that hard grain sugars are usually dryer. Whether their hardness makes them easier to dry, or whether they are hard because of the thoroughness with which they have been dried, are questions which we have not attempted to answer. As regards the hygroscopic nature of sugars we will have more to say under a subsequent text.

THE INFLUENCE OF BAGACILLO UPON SUGAR DETERIORATION.

There seems to be a growing conviction in the minds of the Cuban sugar manufacturers that one of the principal causes of sugar deterioration is the presence of small particles of cane fibre (known as bagacillo in Spanish) in sugars. Browne[1] has shown that a comparative study of the amounts of this material occurring in good keeping and deteriorated samples of sugar tends to lend support to this claim. Thus he finds an average of .07% bagacillo in sugars which kept well, and an average of

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[1] For a very thorough discussion of "The relations between grain size and surface film," the reader is referred to Otto Rahn's valuable work on "The Bacterial Activity in Soil as a Function of Grain Size and Moisture Content." Michigan Technical Bulletin No. 16.

0.16% in sugars which deteriorated. He does not, however, believe that it is a direct cause of the deterioration, but thinks it merely an indication of careless methods of manufacture, which in themselves are conducive towards deterioration.

The writer has made a number of experiments upon the influence of the addition of bagasse particles upon the deterioration of sugars. In every case where the conditions of the experiment precluded the influence of moisture retention by the bagasse the results were negative. In the writer's opinion bagacillo only influences sugar deterioration by the absorption and retention of moisture, by means of which a moist zone is created in which deterioration can take place. No importance need be attached to the theory of the bagacillo acting as a carrier of infection, for the most dangerous infection of sugars is introduced while it is in the centrifugal. The bagacillo, on the other hand, originates at the mill end of the factory process.

PROVIDING THE PROPER STORAGE CONDITIONS FOR SUGAR.

The prevention of sugar deterioration not only necessitates the manufacture of sugars whose molasses films conform in density to the factor of safety, but also upon the maintenance of this density. Applied to industrial conditions, this means that sugars must be stored in a relatively dry atmosphere. When we consider this question in its specific relation to Louisiana conditions we may disregard the question of the influence of certain impurities upon the hygroscopic nature of sugars. In this climate we need not consider the influence of glucose[1] or of certain ash constituents upon the moisture absorbing power of sugars. Where the humidity is so high as it is here, the operation of these influences is by no means necessary for the absorption of moisture by sugar. The writer has frequently observed grains of coarse standard granulated sugar almost completely deliquesce when exposed to the atmosphere on a very humid day. In this case we can hardly attribute the absorption of moisture to the hygroscopic properties of the sugar, but preferably to the pressure

[1] Deerr and Norris, The Deterioration of Sugar on Storage, Hawaiian Bulletin No. 24.

of atmospheric moisture and its resulting condensation upon solid particles. Under these conditions good warehousing is essential to the satisfactory keeping of sugars. Of the type of warehouse in which a dry atmosphere can be most effectively and most economically maintained not much needs to be said. Provision should be made for drying the warehouse when the humidity increases beyond a certain point. It is well to have concrete floors, but the sugar should not be piled directly thereon, but a false floor of boards with an air space between the two floors should be provided.

The changes in the composition of sugar due to the absorption of moisture are of great importance in connection with the sampling of cargoes for valuation according to polarization. The relative moisture absorbing powers of different types of sugar, under different conditions of atmospheric humidity, have been exhaustively studied by Browne.[1] The following table, taken from his "Handbook of Sugar Analysis," shows the gain or loss in the moisture of different sugars at 100% and at 60% relative humidities.

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[1] Handbook of Sugar Analysis, p. 7.

TABLE II.

Kind of Sugar	Grain	Polarization	Moisture in Sugar	Gain first hour 100% humidity	Change first hour 60% humidity	Total change at point of equilibrium	Humidity at equilibrium	Residual moisture at equilibrium
			Per Cent.	Per Cent.	Per Cent.	Per Cent.	Per Cent.	Per Cent.
Granulated	Fine	99.85	0.10	1.78	+ 0.03	+ 0.01 (2 hrs.)	56	0.11
Peruvian	Large	98.40	0.35	1.09	− 0.09	− 0.14 (4 hrs.)	56	0.21
Porto Rican	Medium	96.40	1.31	1.40	− 0.54	− 0.73 (2 hrs.)	62	0.58
Philippine mats	Fine	87.45	3.12	1.80	− 0.68	− 1.25 (6 hrs.)	56	1.87
Cuban molasses	Large	82.75	4.85	1.12	− 1.00	− 2.42 (24 hrs.)	59	2.43

Handbook of Sugar Analysis pg. 7. C. A. Browne.

It will be noted in the above table that the samples of fine grain sugars absorbed more moisture than those of large grain. In discussing the absorption of moisture by sugars under excessive humidity, Browne states that no relationship can be traced in the above table between the composition of the sugar and rate of absorption. However, when the grains are compared it will be found that the smallest grain sugars have the largest absorptive powers. With regard to the evaporation of moisture from sugars, under diminished pressure, the author referred to states that here a very definite relationship exists between composition and rate of evaporation. The rate of evaporation being proportional to the initial moisture content, and the amount of moisture remaining in the sugar at the point of equilibrium, is a function of the hygroscopic quality of the nonsugars.

Prinsen Geerligs,[1] who has made an extensive investigation of the rate of moisture absorption by different sugars, reaches the following conclusion:

Refined sugars, and very high grade raw sugars, absorb moisture at a relative humidity of 80 or over, when the temperature is between 24 and 33°C. The limit for white first sugar is 75, 70 for refinery crystals and molasses sugar, and between 70 and 75 for seconds, according to quality.

In the following table the average mean temperature, in degrees centigrade, is given for nine months of the year in Louisiana, also the average relative humidities for the respective months. The humidities from January to August are for the year 1917, which was unusually dry from March until July. The humidities given for these months are therefore lower than in normal years.

[1] Cane Sugar and its manufacture, pp. 298, 299.

TABLE III.

Table Showing Average Mean Temperature (Centigrade Degrees) and Average per cent Humidity for the Months of the Grinding Season in Louisiana, and the seven months following thereafter.

Month	Mean Temperature	% Humidity
October	20.8	79
November	16.6	68
December	14.5	81
January	15.6	81
February	15.2	78
March	19.0	72
April	19.70	68
May	21.80	63
June	26.00	65
July	27.72	74

From the table it will be seen that conditions of temperature and humidity in Louisiana make adequate warehousing essential to the prevention of sugar deterioration in storage.

THE COLD STORAGE OF SUGARS.

It has sometimes been suggested in connection with the question of warehousing sugars, that the principle of cold storage should be combined with that of dry storage. This, in fact, has been seriously considered in connection with the warehousing of the plantation granulated sugar output of one of the large white sugarhouses in this State.

In the following table are the results of a series of experiments upon the relative deterioration of sugars stored in an incubator in a refrigerator and at room temperature respectively.

TABLE IV.

**Experiment upon Influence of Temperature upon Sugar Deterioration.
Period of Incubation, one month.**

Sample	% Moist.	Sucrose		% R. S.	Dry Basis		Factor	Temp.
		S. P.	S. C.		S. P.	S. C.		
Incubator	3.72	91.15	92.36	2.63	94.67	95.92	.420	34°C
Room Temp.	3.30	90.60	91.81	2.94	93.69	94.94	.351	—
Refrigerator	3.45	94.35	94.85	0.352	97.72	98.23	.610	Mean 20°C
Incubator	1.90	94.95	95.65	—	96.77	97.40	.376	—
Room Temp.	0.50	95.60	96.46	—	96.00	96.90	.113	—
Refrigerator	1.42	95.70	96.54	—	97.78	97.93	.327	—
Room Temp.	2.44	92.90	93.85	2.63	95.22	96.09	.343	—
Refrigerator	2.60	95.60	96.27	1.00	98.15	98.83	.590	—

It will be noted that all of the samples contained sufficient moisture to cause them to deteriorate. However, in every case the refrigerator stored sample deteriorated very slightly as compared to the others. This could only be attributed to the temperature at which the respective samples were stored. The mean temperature of the refrigerator was about 20°C., while the temperature of the incubator was 34°C. The experiment was carried out during the summer months, so that the room temperature was in some cases more favorable for the development of micro-organisms than that of the incubator.

PRECAUTIONARY MEASURES FOR THE PREVENTION OF THE INFECTION OF SUGARS WITH DETERIORATIVE MICRO-ORGANISMS.

While no very conclusive data has as yet been obtained upon the relations between the degree of infection of sugars and their rate of deterioration in storage, yet more and more stress seems to be laid upon the importance of cleanliness in the factory as a preventive measure. Not only so, but wherever the maxim of cleanliness is applied, gratifying results appear to follow. In Hawaii, losses from sugar deterioration seem to have been about entirely eliminated, and there the correction of this trouble is generally believed to have been effected by improving the cleanliness in the factory.[1] Although the writer has been one of the greatest advocates of cleanliness in the factory as a preventive measure against sugar deterioration, he has found it difficult in the light of previous investigation to explain the beneficial effects of this practice. So long as spore-forming bacteria were credited with playing the principal role in the deterioration of sugars, it was difficult to understand how cleanliness could eliminate or even reduce the infection of the products. Since the spores of these bacteria could successfully withstand all of the heating processes incident to manufacture, their elimination was a matter of impossibility, and a material reduction in the initial number of them in the finished sugar could only result from an almost perfect clarification. How, then, could cleanliness eliminate the cuasative agencies in sugar deterioration?

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[1] See the report of Hawaiian Sugar Planters' Association, 1913.

In connection with the consideration of the question of the importance of preventing the infection of sugar with micro-organisms, it is interesting to note the recommendation of Kamerling's[1] that all bags and material used in the packing of sugars should be disinfected. For this purpose he recommended the use of a hot one per cent solution of carbolic acid. The mats and bags were dipped into this solution and dried. Results reported in Prinsen Geerlig's Cane Sugar and Its Manufacture, from experiments on the comparative rate of deterioration of disinfected and non-disinfected samples, show that no benefit was derived from this method of treating the bags. These results were to have been expected since in all probability freshly made sugars are much more likely to carry such infection than is packing material.

To determine the effect of heating upon the deterioration of sugars in storage, the following experiment was conducted. A 96-test sugar was placed under moist chambers and allowed to become very moist. The sugar was then divided into twelve equal portions, of 200 grams, and put in Erlenmeyer flasks. The flasks were inoculated with several cultures of mould, then plugged with cotton, and heated to the temperatures shown in the table.

[1] Cane Sugar and its Manufacture. Prinsen Geerligs, p. 292.

TABLE V.

Experiment Showing Influences of Heat on Keeping Quality of Sugar. One month Incubation.

Treatment	% Moist.	Sucrose		Sucrose Dry Basis		% R. S.	Factor
		S. P.	S. C.	S. P.	S. C.		
Control	4.00	90.3	91.23	93.98	94.91	2.45	.412
I 5′ @ 60°	3.90	90.7	91.71	94.38	95.39	2.69	.419
II 10′ @ 60°	3.79	90.65	91.75	95.36	96.46	2.77	.405
III 5′ @ 65°	3.89	90.2	91.39	93.85	95.04	2.39	.396
IV 10′ @ 65°	3.71	90.7	91.79	94.19	95.28	2.43	.398
V 5′ @ 70°	3.87	90.5	91.47	94.14	95.11	2.60	.407
VI 10′ @ 70°	3.82	90.4	91.23	93.99	94.82	2.26	.397
VII 5′ @ 75°	3.03	91.0	91.86	93.84	94.70	2.16	.336
VIII 10′ @ 75°	3.73	91.1	91.94	94.72	95.56	2.46	.419
IX 5′ @ 80°	3.80	91.4	92.26	95.01	95.87	2.13	.441
X 5′ @ 85°	3.74	91.7	92.26	95.26	95.82	2.10	.450
XI 5′ @ 90°	4.36	91.7	92.34	95.88	96.52	1.81	.525

Original Analysis —
Dry Basis — S. P. = 96.89
S. C. = 97.23

The results of the above table show that heating the sugar for five minutes at 90°C. decreased its deterioration greatly, as compared with the unheated control. Also at 80° and 85° for five minutes the deterioration was much less than in the control flask.

More recent investigations, the results of which have been reported in a previous part of this bulletin, show that bacteria play an almost insignificant role in the average case of sugar deterioration. Moulds are now known to be the most dangerous of all the micro-organisms occurring in sugar. Furthermore, they have a comparatively low thermal death point. As an example, Thom[1] has shown that in the flash process of pasteurization, where milk is heated to 165°F. (73.9°C.) for a period of 30 seconds the spores of most of the mould species were killed. At

175°F. (79.5°C.) only occasional spores developed. When the heating was performed in dry air at a temperature of 30 seconds, at 200°F. (93.3°C.) seven out of twenty-four forms of aspergillus were destroyed.

In the light of these results, the elimination of moulds from sugars seems entirely feasible. On the contrary, it can be well understood that a heavy initial infection of sugars with these spores indicates a condition of uncleanliness existing around the centrifugal which could be easily and profitably corrected. In connection with these facts, Shorey's[1] recommendation for covering the centrifugals, and for using steam for washing sugars, seems to offer a thoroughly practical method for the elimination of one of the most dangerous group of micro-organisms associated with sugar deterioration.

The possibility of decreasing the deterioration of sugars, by pasteurization, is not merely a matter of inference. From the results shown in the above table the writer credits to the effect of such pasteurization the large number of negative results obtained from sugars which were heated to 90°C. for one hour before being inoculated with bacteria. At that time it was not so fully known that bacteria can only cause the deterioration of very moist sugars, so the negative results were obtained in most cases from samples having a factor of .400 to .45.

CONCLUSIONS.

(1) Sugar deterioration is caused by moulds, torula, and in some cases by bacteria.

(2) The mould group includes aspergillus fumigatus, a blue aspergillus, and floccose strains and aspergillus pencilloides.

(3) The moulds constitute the most dangerous group of micro-organisms in sugars, on account of their strong inverting power, their ability to exercise this power in highly concentrated sucrose solutions, and in solutions of varying reaction; also on account of their ability to develop on medium very deficient in nutrients.

(4) The torula are of constant occurrence in sugars. While they are active in destroying reducing sugars, under widely

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[1] Effect of Pasteurization upon mould spores. Journal of Agricultureal Research, Vol. 6 No. 4.

varying conditions, their ability to invert sucrose is comparatively weak, and only exercised under especially favorable conditions.

(5) The bacteria can only cause the deterioration of sugars which have absorbed a comparatively large amount of moisture, and which have as a consequence a very low solid non sucrose moisture ratio.

(6) The "Factor of Safety" of the Colonial Sugars Company of Australia is a remarkably good criterion of the keeping quality of sugars. Its validity is believed to be due to the fact that it indicates a certain density of the molasses films surrounding the crystals of the sugar. It is of less value in predicting the behavior in storage of washed 96-test sugars. It is inapplicable to white sugars, because in such cases, it is the total moisture that must be considered since the density of the film cannot exceed the maximum concentration in which micro-organisms can destroy sucrose.

(7) Cleanliness in the sugar factory, and particularly around the centrifugals, offers a partial means for eliminating the infection of sugars. The use of covered centrifugals, and of steam as a substitute for wash water is to be recommended.

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Agricultural Experiment Station

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BATON ROUGE

Blood-sucking Insects as Transmitters of Anthrax or Charbon

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Blood-sucking Insects as Transmitters of Anthrax or Charbon.

By Harry Morris

Anthrax or Charbon is one of the common live stock diseases in certain sections of Louisiana. Sporadic cases of the disease occur during the greater part of the year, but enzootics always appear during the warm summer months. Several theories have been given concerning these outbreaks, namely, previous infection, favorable climatic conditions, neglect in the proper sanitary care of anthrax carcasses, blood-sucking insects, and many others.

In work carried on in the Louisiana Experiment Station for the past three years an attempt has been made to find out the relation between certain blood-sucking insects and the transmission of anthrax. In the past a few articles have been published concerning the transmission of anthrax by biting flies.

In the review of literature on anthrax very few data have been given concerning the transmission of this disease by the biting of blood-sucking insects. Schuberg and Kuhn[1], in 1912, transferred anthrax infection from a cadaver to a living animal through the biting of *Stomoxys calcitrans.*

The most extensive research work along this line has been carried on by Mitzmain[2], who succeeded in transmitting anthrax through the bites of *Stomoxys calcitrans* and *Tabanus striatus.*

In Louisiana, Dalrymple and Dodson[3], as early as 1900, gave a record showing the relation of the density of the blood-sucking insects to the outbreak of anthrax. But while they were more or less positive in their opinions, based upon what seemed strong circumstantial evidence, they did not submit any direct proof at the time of transmission of infection by the flies.

After observing several enzootics of anthrax in Louisiana it appeared that some unknown factor was playing a prominent part in the continuance and spread of this disease. For that reason a study of some of the most common blood-sucking insects was undertaken in the pathology laboratory of the Louisiana Experiment Station.

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[1] Schuberg and Kuhn. Ueber die Uebertagung von Krankheiten durch einheimesche stechende Insekten. Arb. a. d. kais. Gesundheitsamte (1912), Heft 2, Bd. XL, 209-234.

[2] Mitzmain, 11. Summary of Experiments in the transmission of anthrax by Biting Flies, U. S. Public Health Service, Hygienic Laboratory Bulletin No. 94 (1914).

[3] Dalrymple, Louisiana Bul. No. 60 (1900).

The following article is a report of the work to date. Many phases of this subject have not been touched in this report. It is hoped that the results given will assist in the sanitary control of anthrax in Louisiana.

The purpose of the experiments was to test the possibility of anthrax transmission through the biting of blood-sucking insects. As enzootics of anthrax occur during the prevalence of these insects, it was considered possible that the anthrax bacilli might be carried on the proboscis of an insect to healthy animals after having fed upon an animal sick with anthrax. All experiments were conducted with experimental animals under as nearly natural conditions as possible. Pure cultures of anthrax spores were used in producing the disease.

The flies used in these experiments were the horn fly, *Haematobia irritans L.,* and a horse fly, *Tabanus sp.* This horse fly was sent to the U. S. Bureau of Entomology to be identified. Mr. Frederick Knab reported that the species was *Tabanus sp.* near *nigrovittatus* Macq., apparently undescribed.

The mosquitoes used were *Psorophora (janthinosoma) sayi* D. &. K. and *Aedes sylvestris* Theob. These determinations were also made by Mr. Knab.

TECHNIQUE.

Guinea pigs were inoculated with a loop of anthrax spores injected subcutaneously or into the abdominal cavity. These inoculated guinea pigs were used as sources of infection except in one experiment where a sheep was used. The same strain of anthrax was used in nearly all the experiments and proved to be very virulent. Death would occur in adult guinea pigs twenty-eight hours after inoculation or sooner in younger animals.

The Horn fly is one of the most predominant of all the species of blood-sucking flies. It is prevalent during the greater part of the year, and is found, during the summer months, in swarms feeding upon cattle, more especially upon animals with dark coats, to evade detection.

The flies were collected in a net from the black cattle on the

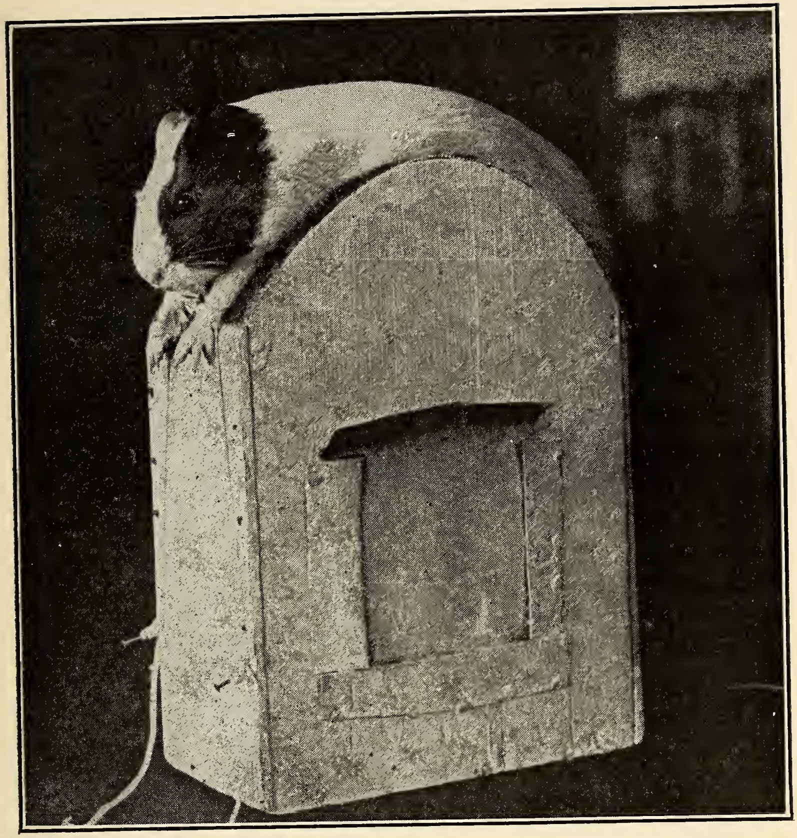

Fig. 1—The cage in which horn flies were kept while feeding upon a guinea pig.

Experiment Station farm. Very little difficulty was experienced in collecting the Horn flies in this manner, and very few other species of flies were found among the large number collected. But, as with other workers, great difficulty was experienced in keeping the flies alive in capitivity, due to the fact that, if left unfed for twelve hours they would practically all die. Frequent feeding upon a living animal was found necessary if they were to be kept alive for any length of time.

The Horn flies were confined in a cage (Fig. 1) similar to the one described by Francis.[1]

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[1] Francis, Journal of Infectious Diseases, Vol. XV, page 3 (1914).

This cage was a wooden box with curved open top covered with fine wire screen, as shown in the illustration. The number of flies used in a cage would vary from one hundred to three hundred.

The guinea pig which had been previously inoculated with anthrax was tied on the cage and allowed to remain for one minute. Then, after removing the animal, the screen was disinfected with a 1-1000 bichloride of mercury solution to eliminate the danger of infecting the healthy guinea pigs through skin lesions. The healthy guinea pig was then fastened on the cage and allowed to remain from one to three minutes. The horn fly will usually feed from three to six minutes. By this method the feeding was interrupted, being started on one animal and continued or completed on another.

The length of time which elapsed between the time of feeding on the infected animal and its death was noted in every case in order to determine at what period in the development of the disease virulent organisms could be transferred by the flies. The animals used were in different stages of the disease, ranging from six hours before death to thirty minutes after death.

In some experiments the hair was removed from the abdominal surface of the guinea pigs, but this was not necessary because it did not seem to affect the percentage of infections produced.

After death all guinea pigs were posted and bacteriological examinations made of the blood taken from the spleen. All doubtful cases were cultured and animal inoculations made to prove results.

The results of these experiments are given in Table 1:

TABLE 1.

Anthrax Transmission by the Horn Fly.

Length of Time from Death of Infected Animal.	Number of Guinea Pigs Bitten by Flies.	Number Dying With Anthrax.	Percentage of Infection.
Six hours before death	10	0	00
Five hours before death	18	0	00
Four hours before death	17	2	11
Three hours before death	15	3	20
Two hours and thirty minutes before death	15	4	26
Two hours before death	14	3	21
One hour and thirty minutes before death.	16	5	31
One hour before death	20	8	40
Thirty minutes before death	22	12	54
Fifteen minutes before death	20	10	50
Five minutes before death	20	11	55
At death	15	5	33
Fifteen minutes after death	10	1	10
Thirty minutes after death	12	0	00

The above table shows that the flies feeding upon an infected guinea pig four hours before its death transmitted the disease to healthy pigs. In only one instance was the same result obtained by the flies feeding upon an infected carcass. The results indicated that the greatest number of transmissions took place a short time before the death of the infected host. This was due to the fact that anthrax bacilli become generalized in the circulation of the animal a short time before death. The horn fly would not feed upon the infected carcass of a guinea pig satisfactorily.

In some cases the internal form of anthrax was produced, but in the majority the external or carbuncular form was most common. The guinea pig died anywhere from forty-eight to seventy-two hours after being bitten by the flies.

The transmission of the disease seemed to be mechanical, the anthrax bacillus being carried from the infected host to the susceptible animal on the soiled proboscis of the fly.

A few experiments were conducted in which a period of time elapsed between the feeding on the infected host and the susceptible animal. The results were negative.

In another experiment an infected sheep was used as the source of infection. The same technique was used as in the preceding experiment. Each cage contained about one hundred and fifty horn flies. The flies were allowed to feed for one-half minute upon the abdominal surface of the sheep, after which the screen was disinfected with a 1-1000 solution of bichloride of mercury. Immediately the flies were permitted to feed upon the body of a healthy guinea pig. The results are given in Table 2 below:

TABLE 2.

Anthrax Transmission by Horn Fly from Sheep to Guinea Pig.

Length of Time from Death of Infected Animal.	Number of Guinea Pigs Bitten by Flies.	Number Dying With Anthrax.	Percentage of Infection.
Thirty minutes before death	2	1	50
Fifteen minutes before death	2	2	100
At death	3	3	100
Fifteen minutes after death	2	2	100

The table shows that eight out of nine pigs died of anthrax. No doubt the high percentage of infection was due to the large number of anthrax bacilli being present in the peripheral blood of the sheep.

Experiment With Horsefly.

The horsefly, *Tabanus sp.*, was collected, during the middle of the day, from horses and cows in the fields just north of Baton Rouge. The fly appeared in great numbers during the latter part of August and through the month of September, 1915. This species was used because the flies were collected with little difficulty and while in captivity bit like fiends when placed on any living animal. The host gave evidence of great pain when the fly was feeding and quite often a drop of blood would escape after the fly had finished feeding.

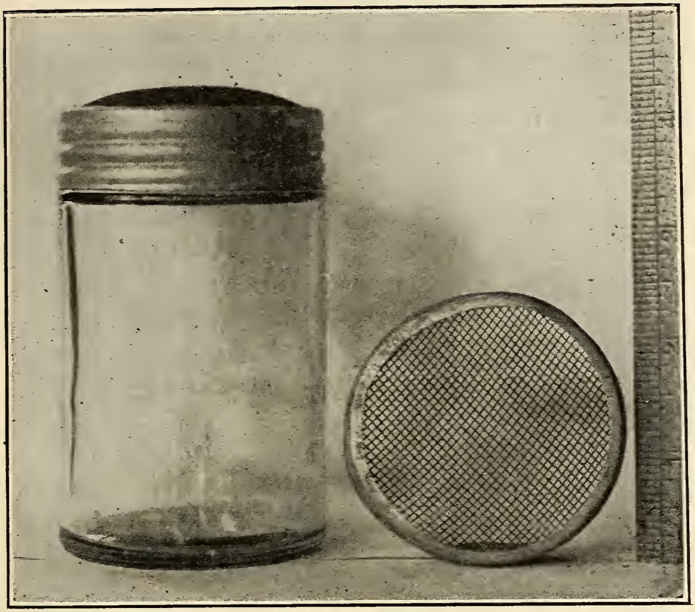

Fig. 2.—Glass jar with screen top used in horse fly and mosquito work.

METHOD.—Small glass jars, as shown in the accompanying illustration, Figure 2, with wire gauze covering, were used as retainers for the flies. One fly was placed in each jar. Several guinea pigs, including the infected one, were secured on small tables with the abdominal surface up. The screen covering the jar was placed against the abdominal surface of an infected pig in the last stages of the disease. Just as soon as the fly commenced feeding the jar was removed and placed upon a healthy guinea pig, where the feeding was completed. If the screen covering of the jar came in contact with blood from the infected animal the metal cap was removed and replaced with a clean one before feeding upon the healthy animal. This was done to avoid inoculation through skin lesions. Table No. 3 gives the results of these experiments.

TABLE 3.

Anthrax Transmisson by the Horsefly.

Length of Time from Death of Infected Animal.	Number of Guinea Pigs Bitten by Flies.	Number Dying With Anthrax.	Percentage of Infection.
Four hours before death	6	1	16
Three hours before death	6	1	16
Two hours before death	5	1	20
One hour and thirty minutes before death.	6	2	33
One hour before death	8	3	37
Fifty minutes before death	5	3	60
Forty minutes before death	6	3	50
Thirty minutes before death	5	3	60
Twenty minutes before death	5	4	80
Ten minutes before death	6	5	83
At death	6	6	100
Five minutes after death	6	1	16
Twenty minutes after death	8	0	00

The above table shows a rather high percentage of infections produced by the method of interrupted feeding. The carbuncular form of anthrax was produced in the majority of cases. The flies would not feed satisfactorily on the carcass of a guinea pig.

Under field conditions another species, a black horsefly, *Tabanus atratus* Fab., sometimes feeds upon carcasses thirty minutes or more after death. Virulent cultures of anthrax have been obtained from the bodies of these flies which had been feeding upon the carcass of an animal that had died of anthrax. These flies could not be induced to feed in captivity, although a great number were collected and different technique used.

It is quite common during the fly season to see blood dropping from the bellies of horses and mules, due to the biting of different flies of the genus Tabanus. In this manner anthrax could be spread from animals suffering with the disease.

EXPERIMENTS WITH MOSQUITOES.

The mosquitoes used in this work were collected near Baton Rouge. *Psorophora (janthinosoma) sayi* D. & K. were found in great numbers in the low-wooded lands along the Mississippi River. They did not like to come into the hot sunshine, but were easily captured on the body of a horse driven into the woods. The animal showed evidence of great pain by their bites. Individuals of this species were easily recognized on account of the bobbing of their third pair of legs through the air while feeding.

The other species of mosquito, *Aedes sylvestris,* Theob, was very common in Baton Rouge during the summer of 1915, and could be collected while feeding upon man or animal. They bit voraciously, and were very annoying to live stock, even the thick skin of a cow could be punctured with apparent ease.

METHOD.—The same technique was used as with the horsefly. The mosquitoes were retained in the same jars (Fig. 2) and allowed to feed through a fine copper gauze. The interrupted method of feeding was used and from one to five mosquitoes were allowed to feed upon each pig. No difference could be noticed in the number of transmissions by the different species. All results are combined in Table No. 4, below:

TABLE 4.
Anthrax Transmission by the Mosquito.

Length of Time from Death of Infected Animal.	Number of Guinea Pigs Bitten by Flies.	Number Dying With Anthrax.	Percentage of Infection.
Four hours before death..................	3	0	00
Three hours and thirty min't's before death	3	0	00
Three hours before death.................	6	1	16
Two hours and thirty minutes before death	7	2	28
Two hours before death..................	7	2	28
One hour and thirty minutes before death.	8	2	25
One hour before death....................	10	3	30
Forty minutes before death..............	5	3	60
Thirty minutes before death.............	10	5	50
Twenty minutes before death............	6	3	50
Ten minutes before death................	9	6	66
At death	10	7	70
Ten minutes after death.................	8	1	12
Thirty minutes after death..............	6	0	00

The above table shows that the anthrax was transmitted through the biting of mosquitoes for a period of three hours before until ten minutes after the death of the infected host. As with the flies, the greatest number of infections was produced just before or at the time of death of the infected guinea pig. The mosquitoes would fede upon the carcass of a guinea pig for only a few minutes after death. Both the internal and carbuncular forms of anthrax were produced.

Examination of Insect Excrement for Anthrax Bacilli.

The subject of fecal contamination was also studied in conjunction with the transmission experiments described in this work.

The same bottles were used for retainers for the insects as in the previous experiment (Plate 2). It was first necessary to determine the presence of anthrax bacilli in the blood of the infected host before permitting the experimental insect to feed. The following method was adopted:

METHOD.—A mosquito or fly was allowed to feed upon the infected host until engorged with blood, then killed and the blood pressed from the body upon a clean slide. The blood smear was stained in the usual manner and examined under the microscope. If anthrax bacilli were present in large numbers other experimental insects were allowed to feed upon the same area on the infected host. Feeding was a very simple operation while working with the horsefly and mosquitoes, as they would feed about every second day. But it was almost impossible to induce single horn flies to feed while confined in a bottle. This presented a serious problem because this fly would not live for any length of time unless fed upon the blood of a living animal. To overcome this difficulty in feeding it was found necessary to place a dozen flies in a bottle and then all would feed immediately when the screened top was placed in contact with a living animal. After feeding upon an infected guinea pig, the flies were anesthetized and each put into a sterile bottle and permitted to deposit upon the glass of the bottle.

The fresh fecal deposits were cultured each day by removing the ''specks'' from the surface of the glass by means of a heavy platinum needle, and cultures made in agar dilution plates. After incubation for twenty-four hours at 37.5° C. the cultures were examined and all colonies resembling anthrax were recultured and inoculations made into guinea pigs. Results obtained are given in the table below:

TABLE 5.

Examination of Excrement for Anthrax Bacilli.

Name of Insect.	Time Following Feeding on Infected Host—Hours.	Number Specks Cultured.	Number With Anthrax.
Haematobia irritans	0–3	426	6
" "	3–6	344	5
" "	6–9	230	0
" "	9–12	148	0
" "	12–15	35	0
" "	15–18	25	0
" "	18–24	10	0
Tabanus	1–12	54	2
"	12–24	49	0
"	24–48	48	0
"	48–72	40	0
"	72–96	36	0
"	96–120	30	0
"	120–144	24	0
"	144–168	19	0
"	168–192	10	0
"	192–216	8	0
Mosquito	0–24	250	0
"	24–48	211	0
"	48–72	124	1
"	72–96	54	0
"	96–120	36	0
"	120–144	20	0
"	144–192	8	0
"	192–240	8	0

The above table shows that one thousand two hundred and eighteen ''specks'' from the horn fly were cultured and eleven produced colonies of anthrax. These colonies were found within six hours after feeding upon an infected host. Two colonies of anthrax were found in three hundred and eighteen ''specks'' from the horsefly. One colony of anthrax was found in seven hundred and eleven ''specks'' examined from the mosquito.

The above results would indicate that anthrax bacilli are mostly if not entirely destroyed in the digestive tracts of the above described insects when ingested from the blood of an animal sick with anthrax. The few colonies found might have been produced from contaminated feet and bodies of the insects.

No attempts were made to feed anthrax spores to see if they would pass through the digestive tract. In this work no microorganism was considered anthrax unless it proved virulent for a guinea pig. This precaution was taken on account of the prevalence of pseudo-anthrax in this locality, an organism which is found in laboratory cultures and mistaken for anthrax.

Summary and Conclusion.

Briefly the results of the experiments described above may be summed up as follows:

1. Horn fly, *Haematobia irritans*, and the horse fly, *Tabanus sp.*, are capable of transmitting anthrax by feeding upon a healthy animal after biting an infected one.
2. Mosquitoes, *Psorophora (janthinosoma) sayi* D. & K. and *Aedes sylvestris* Theob., can transmit anthrax in the same manner as flies.
3. The results would indicate that anthrax is not usually spread in the excreta of the above insects after feeding upon the blood of an animal sick with anthrax.

In this report on the subject of anthrax transmission by blood-sucking insects common to Louisiana we have given a summary of the work and results obtained. A great many points of scientific importance have not been touched in this work. A large number of insect pests should be investigated to determine the part played by them in the continuance and spread of anthrax in Louisiana.

The results of this work should prove the necessity of protecting animals sick with anthrax, against the biting of blood-sucking insects, if the disease is to be controlled. They also show the necessity of keeping valuable animals in screened buildings during the outbreaks of this disease.

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BATON ROUGE

Delayed Ripening of Tomatoes Caused By Spraying With Bordeaux Mixture

BY

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Baton Rouge, La.
Ramires-Jones Printing Co.
1918

DELAYED RIPENING OF TOMATOES CAUSED BY SPRAYING WITH BORDEAUX MIXTURE

C. W. Edgerton

In the tomato-growing sections of Louisiana, the foliage of tomatoes is always more or less affected with two diseases, these generally causing a partial defoliation of the plants and sometimes a complete defoliation. The two diseases are the Early Blight, caused by the fungus, *Alternaria solani,* and the Leaf Mold, caused by the fungus, *Cladosporium fulvum.*

The Early Blight may affect all parts of the tomato plant above ground, including the leaves, stems and fruit. The disease first appears in the form of small brown or black spots on the leaves, especially the lower ones. These spots are scattered and inconspicuous at first but later increase in size and number. If the seasonal conditions are favorable, these spots finally cover the leaves and cause their death. Similar appearing spots may also develop on the stems and fruit.

The Early Blight appears in three degrees or conditions of severity and the recognition of these is essential for the understanding of the results of the experiments described in this bulletin. *First,* the disease may be confined to the lower leaves of the plant, causing the death of these but not spreading to any extent to the upper leaves. This is apparently the usual condition in Louisiana, this condition occurring more often than both of the other conditions together. *Second,* the disease may spread from the lower leaves to the upper ones but not to the fruit. This results in a complete defoliation and death of the plants. While the fruits are not specked, they ripen while hanging on the dead plants and are not of the best quality. Such a condition occurred in the spring of 1918 in most parts of Louisiana. *Third,* the disease may spread to all of the leaves and also to the fruit. Not only are the plants defoliated and killed but the fruit is specked and ruined. Such a condition occurred at Baton Rouge in 1914 in the plots that were being used for the spraying experiments described in this bulletin. An extreme form of this condition occurred at Norwood in 1913, when a large percentage of the fruits in the whole district was ruined.

The Leaf Mold also appears in spots on the leaves, but these spots are very different from the ones caused by the Early Blight. The spots are slightly yellowish on the upper side of the leaf, while on the lower side they are covered with a moldy velvety growth. This moldy growth is at first light colored, but gradually turns dark. These spots spread over the affected leaves, finally causing their death. This disease is common in the state and, together with the Early Blight, is responsible for most of the leaf blighting that is commonly observed.

The general appearance of fields affected with the Early Blight, in any of its conditions of development, and the Leaf Mold would cause one to believe that these diseases produce a heavy loss and it has been the general opinion among pathologists that this is the case. As these diseases can be controlled or checked by spraying with some fungicide, such as Bordeaux Mixture, practically all bulletins and other extension literature on tomato diseases have advocated such treatment.

To test out the efficiency and value of spraying tomatoes for the control of these diseases, some tests were carried on through three seasons. The results obtained were interesting, inasmuch as they brought out a factor which is usually overlooked in spraying experiments. While the diseases were readily controlled by spraying with Bordeaux Mixture, this control was not always beneficial to the crop, and in some cases was detrimental. The spraying caused a delay in the ripening of the fruit, and this is a factor which is of extreme importance in those sections where the tomato is grown for the early market.

The experiments were conducted on the Experiment Station farm at Baton Rouge during the seasons of 1914, 1916 and 1917. During this period various weather and disease conditions were encountered, and it would seem that definite conclusions can be drawn from the data obtained. The conditions included one year with a severe infection of the Early Blight with the disease on both the leaves and the fruit, and two years with a heavy infection on the lower leaves but with very little of the disease on the fruit. The Leaf Mold infection was heavy each season.

The spraying was done in the usual manner. Bordeaux Mixture prepared according to the 4-4-50 formula was used in all of the experiments. The number and time of the sprayings are given in the discussion of each experiment. Untreated check plots were left in all cases. Two varieties of tomatoes were used each season, one variety known to be very susceptible to the Early Blight and Leaf Mold, and one variety which is somewhat resistant. The susceptible variety was the Earliana and the resistant variety was either the Louisiana Wilt Resistant or the Globe. The Louisiana Wilt Resistant is a local variety which had been selected for resistance to the Fusarium Wilt disease. The difference in susceptibility of different varieties is often very marked. In the season of 1918, in unsprayed fields, varieties of the Earliana type were completely defoliated shortly after the fruit began to ripen, while resistant varieties, such as the Globe, kept their foliage throughout the season.

EXPERIMENTS IN 1914

The first spraying experiment was carried on in the spring of 1914. This was a season with a very heavy infection of the Early Blight, the disease occurring on both leaves and fruit. The two varieties used in the experiment were the Earliana and the Louisiana Wilt Resistant. One plot was left unsprayed as a check. Another plot was sprayed six times at intervals of ten days, the first spraying one week after the plants were set in the field and the last spraying at the time of the first picking. A third plot was sprayed only twice, at the time of the last two sprayings of the second plot. The results of the experiment are shown in Figure 1 and Table 1. The curves in Figure 1 show the amount of fruit picked each week from the check and sprayed plots, the yield being figured on an acre basis. The total yield of fruit is given in Table 1.

TABLE 1

YIELD OF TOMATO PLOTS IN 1914

Treatment	Earliana	Louisiana Wilt Resistant
	Yields, lbs.	Yield, lbs.
Check	18,930	13,770
Sprayed six times	29,520	14,060
Sprayed two times	24,700	11,950

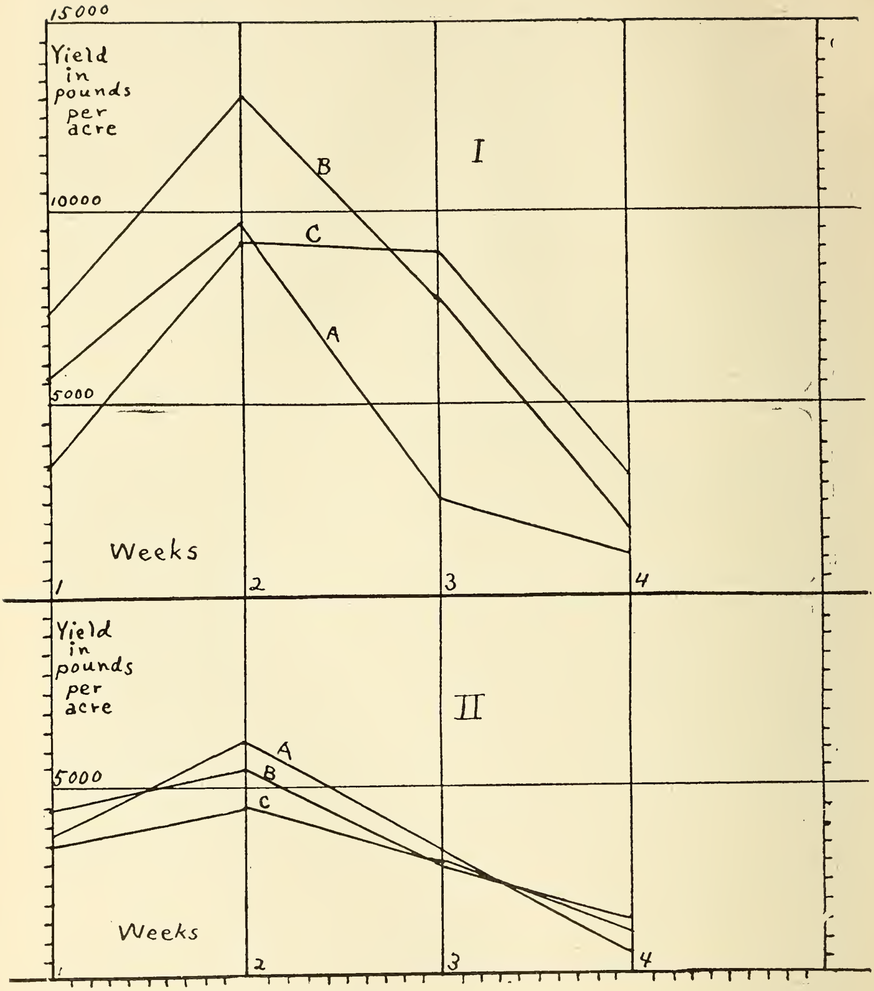


Figure 1. Results of tomato spraying experiments in 1914. I. Variety Earliana. II. Variety Louisiana Wilt Resistant. A. Curves showing the yield per acre at weekly pickings on unsprayed plots. B. Same on plots sprayed six times with Bordeaux Mixture. C. Same on plots sprayed two times with Bordeaux Mixture.

In the tests in 1914 with the Earliana variety, the total yield of the plots sprayed six times was very much larger than of the unsprayed plots. The curve of production in Figure 1 also shows the weekly pickings of the sprayed plots to be greater. Of all the six experiments discussed in this bulletin, this was the only one in which the yield of the first few pickings of the heavily sprayed plots was greater than of the unsprayed. This was due to the heavy infection of the Early Blight on the fruit of the unsprayed plots. A large portion of the fruit on these was ruined by the disease. When the disease threatens to become severe on the fruits, there is no question but what spraying is advisable. The effect of spraying the plants only twice is also very noticeable. The two late sprayings checked the disease and prolonged the picking season for a considerable time.

In the tests with the Louisiana Wilt Resistant variety, different results were obtained than with the Earliana. This tomato is fairly resistant to the Early Blight and there was not very much fruit infection. The difference in yield of the treated and check plots was not enough to be noteworthy. It should be noted, however, that the check plot gave a greater yield at the heaviest picking than the treated ones. While the difference is not very marked, it is noteworthy, as it is in accord with the results of the succeeding years.

EXPERIMENTS IN 1916

The season of 1916 was one with a heavy infection on the lower leaves but with very little on the upper leaves or fruit. The leaves were practically all killed on the lower portions of the plants. The two varieties used in the spraying experiments during this season were the Earliana and Globe. The treated plots were sprayed nine times at intervals of ten days, the first spraying shortly after the plants were set in the field and the last spraying at the beginning of the fifth week of the picking season. The results of the experiment are shown in Figure 2 and Table 2. The curves show the amount of fruit picked each week from the check and sprayed plots, the yields being figured on an acre basis. The total yield of fruit and the percentage that was marketable are given in Table 2.

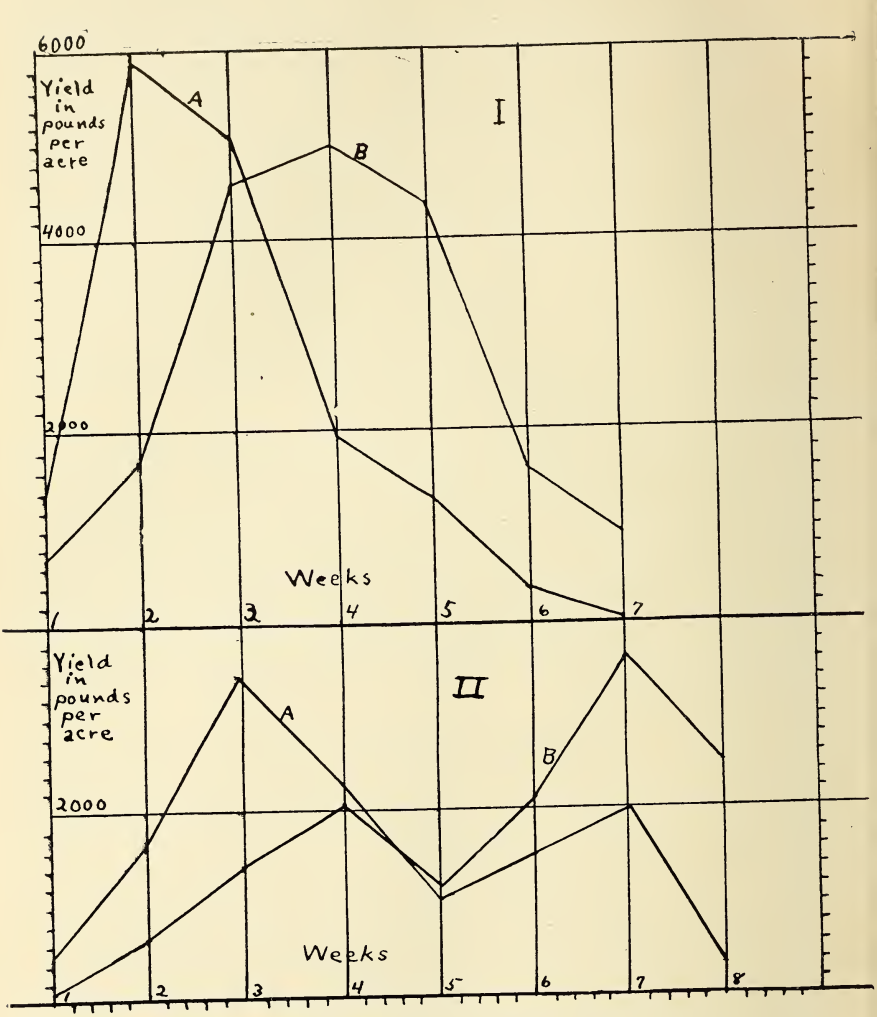


Figure 2. Results of tomato spraying experiments in 1916. I. Variety Earliana. II. Variety Globe. A. Curves showing the yield per acre at weekly pickings on unsprayed plots. B. Same on plots sprayed with Bordeaux Mixture.

TABLE 2

YIELD OF TOMATO PLOTS IN 1916

Treatment	Earliana		Globe	
	Yield, lbs.	Per Cent Market-able.	Yield, lbs.	Per Cent Market-able.
Check	15,865	39	12,500	36
Sprayed	18,585	40	13,680	45

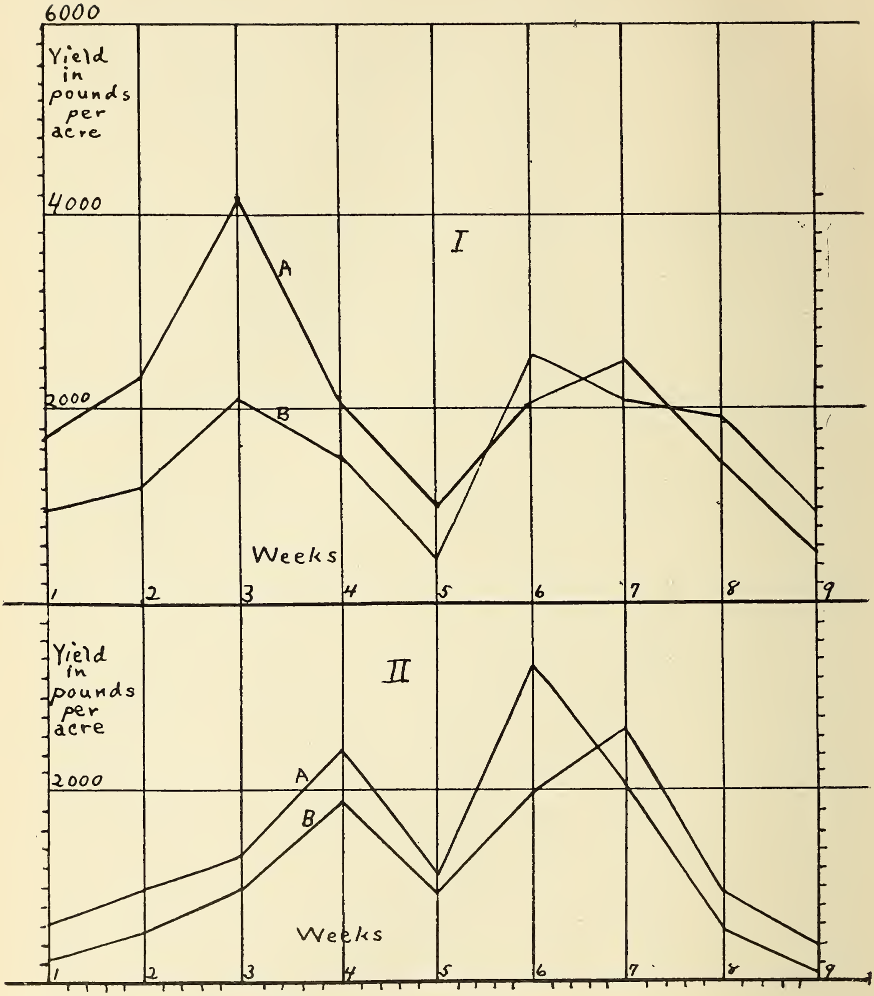
The season of 1916 was a very satisfactory year for the tomato grower; in fact, it was called a very good tomato year. The weather was favorable and the picking season was extended through seven or eight weeks. Perhaps the season could be called more nearly normal than either of the other two years in which the spraying was carried on. On this account it would seem that the results obtained would approach closely to what we would expect for a series of years.

The results of the spraying experiments in 1916 show a slightly greater total yield on the sprayed plots but the difference is not very marked. It should be noted, however, that the difference is greater with the susceptible variety, Earliana, than it is with the resistant variety, Globe.

What stands out most prominently in the 1916 experiments is the delay in the ripening of the fruit caused by spraying and the increase in yield of the unsprayed plots at the early pickings. This is brought out well by the curves in Figure 2. The curves of production show the first few pickings of the unsprayed plots to be much heavier than of the sprayed plots, while the later pickings show the reverse. With the Earliana variety, the second week gave the maximum yield of fruit on the unsprayed plots, while the maximum was not reached on the sprayed plots until the fourth week. To the early market gardener, the yield of the first few pickings is often of more importance than the total yield of fruit.

EXPERIMENTS IN 1917

The third and last set of spraying experiments were run during the spring of 1917. The varieties were the Earliana and Globe, the same as in the preceding year. Although the season was unusually dry, there was a heavy infection of the Early Blight and Leaf Mold on the lower leaves but not much upon the



· Figure 3. Results of tomato spraying experiments in 1917. I. Variety Earliana. II. Variety Globe. A. Curves showing the yield per acre at weekly picking on unsprayed plots. B. Same on plots sprayed with Bordeaux Mixture.

upper leaves or fruit. As a result of the dry season, there was a great deal of Blossom End Rot, but this trouble seemed to be equally abundant on the sprayed and unsprayed plots and so has no particular effect on the results obtained. The treated rows were sprayed five times, the first time about three weeks after the plants were set in the field and the last time at the time of the first picking. It had been the intention to continue the spraying through the picking season, but the dry weather injured the plants so severely that it did not seem worth while. This was unfortunate, as late rains revived the plants, caused new growth and the setting of a second crop of fruit. The early spraying had but slight effect on the ripening of this second crop.

The results of the experiments of 1917 are shown in Figure 3. The curves show the amount of fruit picked each week from the check and sprayed plots, the yield being figured on an acre basis. The total yield of fruit and the percentage that was marketable are given in Table 3.

TABLE 3

YIELD OF TOMATO PLOTS IN 1917

	Earliana		Globe	
	Yield, lbs.	Per Cent Market-able	Yield, lbs.	Per Cent Market-able
Check	17,380	42	12,000	36
Sprayed	13,075	39	10,600	35

Again in 1917, the yield of the check or unsprayed plots at the first pickings was greater than that of the sprayed plots, this, as usual, being more marked with the susceptible variety, Earliana, than with the resistant variety, Globe.

Also in this test, the total yield for the season was greater on the unsprayed plots than on the sprayed. This seemed to be due to the two factors: delayed ripening caused by the Bordeaux Mixture and the abnormally dry weather, working together. In normal seasons, it would have been expected that the curve of weekly production of the sprayed plots would have crossed the curve of the check plots by the end of the third or fourth week, but the drought checked the development of the fruit and prevented this. As the fruit on the sprayed plots developed slower, it was consequently injured more by the dry weather than was the fruit on the unsprayed plots. The drought was broken at the end of the fifth week of the picking season and the plants then developed a new crop. As the spraying had been discontinued at the first picking, the effect of the spray solution on this second crop was greatly reduced; in fact, upon the Earliana it was practically negligible.

DISCUSSION OF RESULTS

Considering the three years as a whole, it is found that in all cases, with the exception of the first year with the Earliana variety, the unsprayed plots have given a considerably larger yield than the sprayed plots during the early pickings. The decreased yield of the sprayed plots seemed to be due to the checking of the various leaf blights which occur on the tomato plants. Under ordinary unsprayed conditions, a considerable amount of the foliage, especially the lower leaves, is killed or badly injured by the Early Blight and Leaf Mold. This partial defoliation of the plants causes a more rapid development of the fruit in the same way as severe pruning will do. Holding this foliage by means of sprays produces a greater vegetative growth and slower development of the fruit.

This delayed ripening of the fruit is of considerable importance in regions where the tomato is raised as an early truck crop. The early fruit usually sells at a much higher price than the late crop and a delay in ripening may mean a considerable loss to the grower. For the grower of early tomatoes, the total amount of fruit is often not as important as is the time when the fruit is produced. To the person who is growing tomatoes in a home garden for his own use or for canning, delayed ripening of the fruit is not of so much importance. In fact, an extension of the picking season may be in many cases desirable.

The difference in the total amount of fruit produced on the sprayed and unsprayed plots was not very marked. During the first experiment, in a year in which there was a heavy fruit infection of the Early Blight, there was a considerable reduction in yield in the unsprayed plots of one variety, but this was partially offset by the results obtained in 1917. In the latter year, the sprayed plots produced less than the unsprayed, due to the excessive dry weather. The percentage of marketable fruit was also not very different on the sprayed and unsprayed plots.

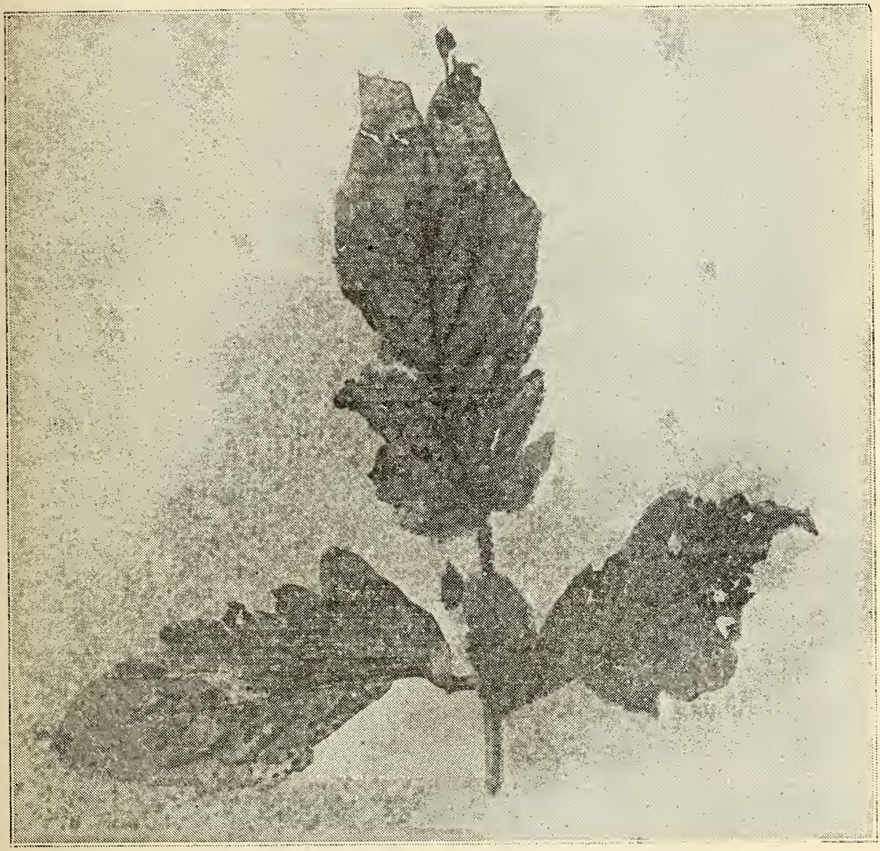


Figure 4. Tomato leaf showing a severe infection of the Early Blight in the early stages.

Considering the data obtained, it seems as if we should modify the recommendations which are generally given for spraying tomatoes, especially tomatoes that are grown for the early market. Heretofore, spraying has generally been advocated in order to control the leaf diseases, but our tests have shown that a limited amount of these troubles is beneficial to the crop, although an excessive infection may be quite harmful. For such years as 1914 and 1918, when the Early Blight was present on both the lower and upper leaves and was severe enough to defoliate the plants, a partial checking of the leaf troubles would doubtless have been beneficial. It would seem that if we could check these diseases to a limited extent, it would be far better than to try to control them. The obtaining of this happy medium, however, may not be as easy as it may seem. It will require a very careful watching of the plants and considerable judgment in the time and number of sprayings.

It would seem best to spray the plants in the seed bed a week or ten days before they are to be set in the field, especially if the Early Blight is present. Often the Early Blight spreads very rapidly in the seed bed, especially if the plants are crowded, and it seems best to have them in as healthy condition as possible at the time of transplanting. The disease is readily checked in the seed bed, usually one application being sufficient.

After the plants are set in the field, they should be watched very carefully. If a severe infection of the Early Blight threatens at any time during the season, it should be checked by spraying with Bordeaux Mixture. It would usually not be necessary to apply this spray until the season was fairly well advanced. The most critical time with the Early Blight is that period beginning about two weeks before the first fruits are ripe and ending when the crop is well made. It is usually during this period that the disease spreads from the lower leaves to the upper ones. If the disease does begin to develop upon the upper leaves to any extent, it should be checked with Bordeaux Mixture. The disease is readily recognized during this stage of its development. The small, dead, blackish to brownish spots (Figure 4) on the upper leaves are very characteristic. As long as the disease stays on the lower leaves, there is no cause for alarm, but when it begins to develop seriously on the upper leaves there is danger of complete defoliation and death of the plants. A few sprayings a week or ten days apart should ordinarily be sufficient to check the disease to such an extent as to insure a crop. In 1914, two late sprayings on the Earliana variety increased the yield over the unsprayed to a considerable extent.

It has been stated on a previous page that the effect of the different diseases on the lower leaves of the plants is similar to that of pruning. Each hastens the development of the fruit. The question naturally arises, Wouldn't it be better to prune the plants and then keep the diseases in check by spraying? There are two serious difficulties with this procedure. *First,* there is considerable expense and labor in thorough pruning and spraying; to keep the plants thoroughly pruned, especially in rich alluvial soils, means a considerable expense. *Second,* pruning is responsible for the spread of the Mosaic disease, another disease which is becoming very serious in Louisiana. As the Mosaic disease cuts down the yield of a plant about fifty percent, the serious spread of the trouble is liable to cause considerable loss. Fields have been seen in which practically every plant was affected with this disease, the heavy infection apparently being due to too frequent pruning. It is not practicable to eliminate all pruning, but too frequent pruning should not be practiced. In regions similar to that at Baton Rouge, one good pruning is generally sufficient.

SUMMARY

1. There are two leaf diseases of the tomato in Louisiana which are very prevalent, these being the Early Blight, caused by the fungus, *Alternaria solani,* and the Leaf Mold, caused by the fungus, *Cladosporium fulvum.* These diseases generally cause a partial defoliation of the plants and occasionally a complete defoliation. The Early Blight may also, under favorable conditions, infect the fruit, and in such favorable seasons the loss may be considerable.

2. Tomato varieties show a great deal of variation in regard to resistance to the Early Blight. Such varieties as the Earliana and June Pink are very susceptible, while such varieties as the Globe and Duke of York are fairly resistant.

3. Spraying with Bordeaux Mixture will check the leaf diseases very satisfactorily.

4. Spraying causes a delay in the ripening of the fruit which is an important factor to the man who is growing tomatoes for the early market. This delay in ripening is from one to two weeks with plants that are kept thoroughly sprayed through the season.

5. Spraying may or may not cause a greater total yield of fruit, this depending upon the season and also upon the seriousness of the infection.

6. The delayed ripening seems to be due to the greater vegetative growth of the plants. The diseases on the unsprayed plants have somewhat the same effect as pruning the plants—each results in hastening maturity.

7. As a result of the delay in ripening, the plants should only be sprayed enough to check the diseases during very serious epidemics. It does not seem advisable to try to control the diseases entirely.

8. It is better to let the diseases partially defoliate the plants than it is to remove this same foliage by pruning. Pruning tends to spread another trouble, the Mosaic disease, which promises to become very serious in Louisiana. Pruning is often desirable, but should not be practiced more than necessary.

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Baton Rouge

The Color of Sugar Cane Products and Decolorization in Factory Practice

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PREFACE.

In Bulletin 161 of this station we presented a summary of work done by previous investigators on the preparation, properties, and use in the cane sugar industry of vegetable decolorizing carbons, and reported the results of laboratory and small scale sugar house tests, undertaken during the grinding season of 1917-18. The object of those experiments was to secure general information on the merits or demerits of decolorizing carbons, principally from an analytical point of view, and to decide whether these carbons remove only coloring matter, as claimed by some investigators, or whether they have a more pronounced purifying effect, as asserted by others. It was found that the latter view is correct, and that these carbons do take out a certain proportion of gums, ash, and nitrogenous constituents, and that they bring about a measurable increase in the purity of the products. Furthermore, the coloring matter is actually removed, and not simply bleached, as is the case when sulfurous acid, hydrosulfites, or similar materials are used.

Our researches on vegetable decolorizing carbons have been continued during the past year, mainly along two different lines. A number of methods for the preparation of such carbons have been studied in detail, and the results of this work will shortly be ready for publication. During the grinding season of 1918-1919 some further tests on the use of these carbons in the sugar house were undertaken, on a larger scale than the year before, and these experiments form the subject of the present article.

The authors gratefully acknowledge the active cooperation and helpful advice received from Mr. W. G. Taggart, Assistant Director, and extend their thanks to all the members of the staff, and to Mr. W. L. Owen, of Penick & Ford Co., for giving us the benefit of their experience.

REVIEW OF LITERATURE ON DECOLORIZING CARBONS FOR 1918.

Since the publication of our last bulletin on the subject of vegetable decolorizing carbons a number of patents for the manufacture of such carbons have appeared. Some of the older processes have been modified, and new ones have been worked out, but no new principle is involved in any of those that have come to our notice. Since all these patents deal more particularly with the preparation of carbons, their discussion has been reserved for our publication on this phase of the subject.

Only three articles on the use of vegetable carbons in the sugar industry have been noted.

Weinrich[1], in reply to Sauer[2], admits that Norit removes, besides coloring matter, also gums and slime, but maintains that this effect is negligible, and may be utilized, in actual practice, only for the treatment of liquors of 99 purity or higher, but not for that of raw sugar washings or cane juice. He also quotes the opinion of an unnamed prominent technologist to the effect that the finely divided state of these carbons causes them to form colloidal solutions in the liquor—unless these be decidedly acid—and thus may do more harm than good. Weinrich and this technologist are also of the opinion that the problem of the revivification of these carbons has not yet been solved. Weinrich believes that better results would be obtained by the use of a vegetable carbon deposited on a mineral skeleton of sufficient hardness so that it may be handled and revivified like bone black.

Prinsen Geerligs[3] reports that the Java factory (Krian), which had made extensive experiments with Norit, had not adopted it for their manufacturing process, apparently on account of the difficulties encountered in revivifying the carbon. He also states that the patentees of Norit had abandoned the idea of using Norit on juices, and now advocated its use for refining raw sugars.

While the two articles quoted are rather unfavorable to the use of vegetable decolorizing carbons on juices, Peck and Adams[4] report excellent results from the treatment of clarified cane juice with a carbon made by digesting final molasses, preferably mixed with Kieselguhr, with concentrated sulfuric acid, and removing the excess of acid by washing with water. In laboratory tests increases in purity of 1.5 to 4.4 points were obtained, and a decolorization of 95% with new carbon, falling gradually to 70% after repeated use. Almost as good results were recorded in small scale experiments with the use of a filter press, and tests on a still larger scale turned out very favorably, in spite of the poor quality of the juices worked with towards the end of the grinding season. Peck and Adams first fill the press with carbon, and then pass the juice through it, and get better results from this procedure than were obtained by the older method of mixing the carbon with the juice, and then pumping the mixture through the press. When the flow of juice becomes considerably retarded, the sucrose remaining in the carbon is washed out, and then the carbon is revivified by passing hot water through it and finally steaming it. When this method becomes ineffective, on account of the accumulation of impurities, the carbon is revivified in the same way in which it is made, by treatment with concentrated sulfuric acid. The authors state that the carbon almost completely removes the flavors peculiar to the raw juice, that the treated juice boils very freely to syrup and to grain, and that practically no scale is formed.

In the writers' opinion, based on the results obtained at this station, vegetable decolorizing carbons can be used successfully on cane juices, and this is corroborated by the work of Peck and Adams. We have had no opportunity to study on a large scale the efficiency of the various methods of revivification, with the use of alkalies or reburning, but the treatment with sulfuric acid, as advocated by Peck and Adams, opens a new perspective. It is not only desirable, but absolutely necessary, that large-scale experiments be made in a sugar factory, to work out the economical side of the question. Any figures that we might arrive at from tests made at the station sugar house, might lead to entirely erroneous conclusions, because they would not be directly applicable to large-scale operations. It would seem very strange that no such factory tests have so far been made in Louisiana, in spite of the great interest displayed by several planters, were it not for the fact that during the past two years no single decolorizing carbon for sugar products has been obtainable in the United

States in sufficient quantities. It is very much to be hoped that, now the war being practically over, this new promising field will at last get the attention it deserves.

PURPOSE OF THE SUGAR HOUSE TESTS MADE AT THE STATION DURING THE GRINDING SEASON OF 1918-1919.

Our recent sugar house tests were undertaken with the object of clearing up some questions which had been left partly or wholly unanswered in the previous experiments. During the campaign of 1917-1918 we were unable to carry out quantitative color determinations in the various products, for lack of proper laboratory equipment. For the same reason we were unable to seek an answer to the question, opened up by Schneller's investigations[5], as to how the iron and the polyphenols found in our cane products affect the color of the same. The solution of this problem was practically reached during the summer, and the grinding season saw us prepared for the work contemplated.

We also wished to gain some further information on the effect of decolorizing carbons on the quality of the various products and the yield of first sugar. The work of 1917 had been done on a very small scale, one clarifier (3,000 lbs.) each of juice being used for the tests with and without carbon, respectively. This had a great advantage from the analytical standpoint, because the two juices which were to be compared, were first thoroughly mixed, before treating one of them with carbon, and we thus had an absolute basis for comparing their composition by analysis. But, on the other hand, the amount of juice used in each case was too small to give us any satisfactory data on comparative sugar yields obtained, since the boiling to grain had to be done in a very small pan, which did not enable us to make the different sugars under comparable conditions. For these reasons we decided to make further tests on our regular sugar house scale, which means the use of at least five clarifiers full of juice to each strike. This furnished us a better basis for determining the relative quantity and quality that may be expected in actual factory operations on the large scale, but had the drawback that the juice used in one run did not have the same composition as that used in the others. It will therefore be necessary, in interpreting the actual results obtained, to make allowance for differences in the quality of the juices used in each run.

STUDIES ON THE COLORING MATTER IN CANE PRODUCTS AS AFFECTED BY DIFFERENT METHODS OF CLARIFICATION AND BY CARBONS.

Prinsen Geerligs[6] points out that in the manufacture of plantation white sugar too much attention should not be paid to the increase in purity effected by a certain method of clarification, and that it is more important to improve physical properties by the removal of color and of colloidal impurities, the quantity of which is not sufficient to affect the purity to any great extent. If this is so—and there seems to be no reason to doubt it—then any process which promises to fulfill these two conditions deserves the attention of the sugar manufacturer. This was our reason for investigating the color of all the different products obtained in the sugar house by the sulfitation white sugar process, including a modification of it, and of those made by using vegetable decolorizing carbon in addition to the usual clarifying agents. At the same time we also carried out a study of the nature and relative quantity of what appeared to us a very important constituent of the coloring matter existing in the products.

The nature and relative importance of the well known coloring matters occurring in the cane and formed during the manufacturing process are discussed in detail in the standard handbooks on white sugar manufacture from cane, by Prinsen Geerligs[6] and by Harloff and Schmidt[7], and a short summary of what they have to say on the subject will suffice here. Considering first the coloring matters of the cane itself, the

Chlorophyll is not of much practical importance, because it does not dissolve in the cane juice, but occurs in it only as a mechanical impurity which is readily removed with the scums.

Anthocyanin is to be considered more seriously. Anthocyanin is a collective name for the compounds which impart varying shades of red, purple, or brown to the rind of certain cane varieties. They are soluble in cane juice, are precipitated by large amounts of lime, but only incompletely by small quantities. It appears that the elimination by lime is not due to a chemical process, but is a case of physical adsorption. Sulfurous acid decolorizes anthocyanin to some extent, but only temporarily. In the carbonation process anthocyanin is completely removed, while in the sulfitation process the proportion eliminated will depend on the quantity of the precipitate formed in the clarification.

Saccharetin, the "incrustating" coloring matter of the cane, is insoluble in cane juice, but, very much like chlorophyll, enters the raw juice mechanically along with fine particles of fiber. As long as the reaction of the juice remains acid, it does not dissolve, but whenever the fiber particles come in contact with lime or another alkali, the saccharetin is dissolved with a yellow color. But even this is not very dark, and the saccharetin as such is therefore not considered of very great importance, especially in acid processes, as are generally used in Louisiana. We shall see, however, that under certain conditions the saccharetin may contribute materially to the coloring matter of the products.

The three coloring matters mentioned are the only ones present in the cane itself that were, some years ago, considered of any importance.

Turning now to the coloring matters formed during the process of manufacture, we shall first consider those produced by the

Effect of high temperatures. Solutions of all the three sugars existing in cane juice darken perceptibly when heated to high temperatures for any length of time. Levulose does this most readily, dextrose less so, and sucrose least. In the presence of other impurities always found in cane products, like neutral salts and nitrogenous bodies, the darkening is more pronounced. The exact nature of the decomposition products is not known, but they usually do not contain true caramel, this latter substance being only rarely formed in factories with modern equipment, and then only under abnormal conditions. They can be reduced in quantity by proper syrup treatment, but any formed in the pan cannot be eliminated as such by any method now known. In slightly acid solutions they do not form as readily as in neutral solutions.

When the reaction of the products becomes alkaline, however, then we encounter one of the worst enemies of white sugar manufacture, the

Glucosates of alkalies and alkaline earths. When a solution containing reducing sugars is heated with lime or a similar alkaline substance, it soon begins to darken and finally becomes dark brown, and even almost black. The reducing sugars are decomposed and converted into acids, known as glucinic and saccharinic acids, which combine with the lime present, and reduce the alkalinity of the solution. Salts of strong bases with weak acids, like organic lime salts, have the same effect as the bases themselves, even though to a smaller extent. The formation of these dark colored products can be entirely avoided by keeping the reaction of the material constantly on the acid side.

The authors already quoted also mention an outside source which may give rise to a dark color in the products, namely,

Iron salts, taken up by the products from the machinery through which they have to pass. Iron has long been known as the source of the dark color of certain beet sugars. Prinsen Geerligs[8] showed that the greyish tint of some cane sugars is likewise caused by iron, and what is more important, that the iron compound was present not only in the molasses adhering to the sugar, but in the crystal itself. He suggested that the iron was in the form of a saccharate. In Louisiana, Shilstone[9] and also Morse[9] called attention to the fact that iron was responsible for the dark color in certain sugars. The former expressed the opinion that, on account of the very small quantities of iron present, the iron compounds themselves must be of an extremely dark color, and suggested iron salts of "organic acids." From further investigations in Java the conclusion was drawn that, as only ferric salts give any trouble, and furthermore, iron does not crystallize with sucrose in an acid medium, any deleterious effect of iron could be entirely prevented by the use of acids, especially such as sulfurous acid, which latter would not only reduce the iron to the ferrous state, but also provide the necessary acidity to keep the iron from entering the sugar crystals.

Later, Langguth-Steuerwald[10], in his researches on saccharetin, found that this compound, as prepared by him, gave a dark coloration with ferric salts, and upon dry distillation yielded pyrogallol. He concluded that saccharetin and its decomposition products were, in conjunction with iron, responsible for the dark color of sugar products.

Taking up this subject in Louisiana, Schneller[5] called attention to the fact that, while saccharetin or products derived from it, could in combination with iron cause a dark color, there exists in cane a constituent which affords a much simpler explanation, because it is soluble in water and in cane juice. Furthermore, this substance must have formed part of the saccharetin of Langguth-Steuerwald, owing to the method of preparation employed by him, and must therefore be at least partly responsible for the iron reaction of this substance. We refer to "tannin", first discovered in cane by Szymanski[11], precisely through its reaction with iron salts, giving rise to a dark green color or precipitate. These results were confirmed by Browne[12], and Schneller was the first to point out that the presence of tannin or similar water soluble polyphenols could easily explain the dark color of cane products. Schneller also called attention to the fact that the presence of aromatic compounds like saccharetin was not confined to the sugar cane, but that they are found in all lignified plant tissues. This subject requires further study, but the fact remains that for the present the tannin of the cane must be considered the most important constituent of cane giving the iron reaction. Schneller suggested that the polyphenol of the cane need not necessarily be a true tannin, but may be of much simpler constitution, as for instance represented by pyrocatechin, which also gives the green iron action. Investigations on the nature of the cane tannin are now in progress.

The polyphenols of the cane assume even greater significance, from the standpoint of the color of raw juices, as has already been pointed out by one of the authors of this article[13]. The muddy green color of these juices has usually been ascribed to the presence of finely divided particles carrying chlorophyll. But the color of the mill juice itself, after the removal of suspended matter, is usually green, and this is simply due to the compounds formed from the polyphenols and the iron dissolved from the mill by the cane juice. This iron is at the beginning in the ferrous state, but a rapid change takes place, owing to the simultaneous presence in the juice of oxidizing enzymes. These were first discovered in cane by Raciborski[14], whose results were confirmed by Browne[15]. The renewed study of this subject at this station has brought out the fact that cane juice contains an oxidase (a laccase), peroxidase, and tyrosinase. These, in connection with the polyphenols and iron, explain all the different colors that a raw cane juice may assume. It was found experimentally that raw juice—which in the sound cane itself is devoid of color—if extracted in the absence of iron, does not turn green, upon exposure to air, but dark brown. This color is due to the oxidation taking place under the influence of the enzymes named on the polyphenols of the cane. The tyrosinase does not act on the polyphenols proper, but on the tyrosin which was found by Zerban[16] to be present in cane juice in very small quantities. This latter reaction plays a very small part, however, in comparison with the effect of the oxidase on the polyphenols. The exact color of a raw juice will depend on the relative quantities of polyphenols and iron. If no iron is present, the color will be brown, but will be more and more greenish with increasing quantities of iron extracted from the mill.

It should be mentioned here that the anthocyanin, mentioned above, also belongs to the polyphenols, and that the color of its solution is darkened by iron salts.

It has further been found that pyrocatechin may be formed by the overheating of sucrose solutions (Lippmann[17]) and also of dextrose solutions (Munk[18]).

With the knowledge which has been gained of the role played by both polyphenols and iron in the color of sugar cane products, we can now summarize what importance may be attached to the different coloring matters, based on their effect upon the color of the various products made in the processes generally used in white sugar manufacture in Louisiana.

The chlorophyll is of no practical importance, as has already been pointed out. Since liquors are always kept acid, and can become alkaline only locally, owing to the action of lime particles—and even this only temporarily—the effect of glucosates can also be largely discounted. For the same reason the saccharetin itself cannot be of much importance, but can become so by giving rise to compounds which give the iron reaction. This leaves then

(1) The effect of that part of the anthocyanin from dark colored canes, which has not been precipitated. This is evidently of some importance by itself and more so owing to its reaction with iron salts.

(2) The effect of the products formed by the action of high temperatures on the sugars in the presence of other impurities. But this effect is much less in slightly acid solutions than in neutral or slightly alkaline media.

(3) The effect of the polyphenols, principally in connection with iron. It is maintained in Java that iron salts are of no great consequence because they can be rendered innocuous by the use of sulfurous and even any other acid. But this is only conditionally correct, because we have found that our products, in spite of being heavily sulfured, do contain a large part of the iron in the ferric form, and also because, if there is any trace of molasses film left on the sugar crystals, the ferrous iron present will be gradually oxidized to the ferric form, and may thus cause a subsequent darkening of the sugar. The effect of other acids is also problematical, because according to Schneller's results the light color produced in sugar products by adding acid, is largely due to a dissociation of the iron-polyphenol compounds; as soon as the acidity is lowered, or even the temperature lowered, recombination immediately takes place.

Since the question of the effect of polyphenols and iron on the color of the products appeared to be of such great importance, we devoted the last grinding season principally to a study of the color, and of the iron and polyphenol content of our products, as made by the usual methods of clarification, and by the same processes with the additional use of a decolorizing carbon.

Plan of the Sugar House Experiments.

The quantity of cane at our disposal permitted us to make five full runs, and a small sixth run. The first of these cannot be compared directly with the others, because the products of the initial run in any sugar house pick up so much foreign material from the unused machinery, tanks and pipes as to become entirely abnormal in many respects. Runs 2 to 5 were used for experiments with carbon on juice, and run 6 for one on syrup. The detailed plan was as follows:

Run 1 (straight sulfitation with high final acidity; without carbon), consisting of five clarifiers. The raw juice was sulfured to an average acidity of 6.1 cc n/10 per 10 cc of juice, limed back to an average acidity of 1.6 cc, heated to a break, and then treated as usual, running the clear juice into settling tanks, and filtering bottoms and scums through a press.

Run 2 (straight sulfitation with high final acidity; with carbon), consisting of five clarifiers. The raw juice was sulfured to an average acidity of 5.6 cc, and limed back to one of 1.6 cc. Then rice hull carbon was added, at the rate of one per cent on the weight of the juice, and mixed well with the juice, which was then brought to the boiling point and filtered in its entirety through a press.

Run 3 (straight sulfitation with high final acidity; without carbon), consisting of five clarifiers. Raw juice was sulfured to an average acidity of 6.2 cc, and limed back to 1.6 cc. The further treatment was the same as in run 1, no carbon being used.

Run 4 (sulfitation-phosphatation with high final acidity; without carbon), consisting of five clarifiers. Raw juice sulfured to 5.6 cc, and limed to as close to neutrality as possible. Then the juice was again acidified with phosphoric acid (U. S. P. grade of 85%), to an acidity of 1.1 cc. It was then clarified like in runs 1 and 3, no carbon being used.

Run 5 (sulfitation-phosphatation with high final acidity; with carbon), consisting of five clarifiers. Raw juice sulfured to 5.4 cc, and limed back to as close to neutrality as possible. Then rice hull carbon was added, 0.7% on the weight of the juice, the latter being acidified at the same time with phosphoric acid to 1.3 cc. The juice was well mixed, brought to a boil, and all of it filtered through the press.

Run 6 (straight sulfitation, with low final acidity; carbon used on syrup), consisting of three and a half clarifiers. Raw juice sulfured to 5.0 cc, and limed to 0.2 cc. Heated to break, and then treated in usual manner. The clarified juice was boiled to syrup. Part of this syrup was treated with 5½% of its weight of rice hull carbon, then the acidity raised 1 cc by addition of phosphoric acid, the mixture boiled and passed through the press. This experiment was not carried further than the syrup, because its quantity was insufficient for making a full strike. The treated syrup was again mixed with the untreated, all of it boiled to grain, and the strike was finished by drawing in first molasses from the other runs. The sugar and molasses from this run are therefore not included in the experiments.

It will be noted that in runs 1, 2, and 3 the generally accepted sulfitation process for making white sugar in Louisiana was adopted, carbon being used in run 2, and left out in runs 1 and 3. In runs 4 and 5 the juice, after being sulfured and limed back to neutrality, was acidified to decided litmus acidity which, according to Cross's work at this station[19], does not cause inversion, and according to the inventors of Norit[20], materially assists the effect of the carbon. Carbon was left out in run 4, and added in run 5.

We had originally planned to use the carbon on the clarified juice, which in our opinion is the proper procedure, especially when the carbon has to be revivified. For this reason the station had purchased an additional filter press large enough to hold the quantity of carbon used with one clarifier full of juice. But it was found impossible to work in this way, because the acute lack of help made it necessary for us to complete the grinding season in the shortest possible time, and to simplify the operations as much as possible. With the procedure originally contemplated, it would have been necessary to wait for the clarified juice from each clarifier to be treated with carbon and refiltered, while in the method adopted the juice could be boiled at once to syrup. Besides more time, more operators would also have been necessary, and not having these we had to resort to the final plan adopted. Moreover, the new filter press was found to act very slowly, on account of its small size, and this was an additional reason for not using it. We would, however, not recommend this procedure in regular work, except in connection with such carbon processes as Sandmann's[21].

The general analysis of the various products of each run is shown in Table I on page 14.

TABLE I.

GENERAL ANALYSIS OF PRODUCTS.

PRODUCTS	Total Solids	Sucrose	Red. Sugars	Purity	Gluc. Ratio
RUN 1.					
Raw Juice	15.9	12.0	1.41	75.5	11.8
Clarified Juice	15.7	11.9	1.40	75.8	11.8
Syrup	61.6	48.8	6.15	79.2	12.6
First Molasses	84.7	52.4	16.3	61.9	31.0
First Sugar	98.22	94.45	1.33	....	....
RUN 2.					
Raw Juice	14.8	10.4	1.61	70.3	15.5
Clarified Juice	14.3	10.2	1.57	71.3	15.4
Syrup	60.4	45.9	7.27	76.0	15.8
First Molasses	89.7	48.6	21.9	54.2	45.0
First Sugar	97.92	90.74	2.78	....	....
RUN 3.					
Raw Juice	16.5	12.2	1.40	73.9	11.5
Clarified Juice	16.2	12.0	1.38	74.1	11.5
Syrup	60.1	47.5	5.97	78.3	12.6
First Molasses	85.0	49.2	19.1	57.8	38.7
First Sugar	98.19	93.72	2.46	....	....
RUN 4.					
Raw Juice	14.6	11.4	0.92	78.1	8.1
Clarified Juice	14.5	11.5	0.95	79.3	8.3
Syrup	57.9	47.4	4.12	81.9	8.7
First Molasses	84.0	54.8	12.7	65.2	23.2
First Sugar	98.09	93.69	1.83	....	....
RUN 5.					
Raw Juice	14.5	11.2	0.90	77.2	8.0
Clarified Juice	14.3	11.3	0.93	79.0	8.2
Syrup	47.8	39.1	3.15	81.9	8.0
First Molasses	86.2	56.1	11.7	65.0	20.8
First Sugar	98.87	95.10	1.53	....	....
RUN 6.					
Raw Juice	14.7	11.3	1.16	76.9	10.3
Clarified Juice	14.5	11.3	1.11	77.9	9.8
Syrup, without carbon	44.6	34.2	4.49	76.7	13.1
Syrup, with carbon	*	*	*	*	*

*Sample lost.

Quantitative Determination of Coloring Matters.

The quantitative determination of the total color, polyphenols, and iron would have been a very difficult task without a special instrument which permitted us to do this work rapidly and with fair accuracy. The whole question resolves itself into colorimetric measurements, since the quantities of polyphenols and iron are so small that they cannot be determined by ordinary methods. Moreover, iron had to be determined in both the ferrous and ferric state, and for this reason the most sensitive colorimetric method for determining iron, that of Eastick, Ogilvie and Lindfield[22] by means of the sulfide, could not be employed. It was therefore necessary to determine the ferrous iron with ferri-ments after addition of ferric iron, could not be used either for cyanide, and the ferric iron with ferrocyanide. The method proposed by Schneller for the polyphenols, colorimetric measure-our purposes, because in some of our products there was already more iron present than could combine with the polyphenols. For this reason we resorted to the delicate reaction of Allen[23], involving the use of ferricyanide and ammonia.

It soon became apparent that ordinary colorimetric measurements would be of very little use, owing to the differences in tint, not only in the total color of the products themselves, but also in the color of the solutions obtained after the addition of the various reagents. The spectrophotometer could, of course, have been used for this work, but the complexity of the operations involved excluded it for determinations of this kind with products which are not stable and whose color readily changes when they are kept for any length of time. The Lovibond tintometer was also not applicable to this work, but satisfactory results were obtained by the use of the Hess-Ives tint-photometer.

This instrument has been described in detail in various journal articles, but a brief outline of the principles upon which it works may not be out of place here. The white, filtered light of an artificial daylight lamp is thrown on two blocks of perfectly white magnesia. These blocks reflect the light by means of a mirror through two corresponding slits of equal width, one of which is entirely open while the other can gradually be closed. The more this slit is closed, the less light will pass through the opening, and the varying width can be read on a scale divided into 100 equal parts. Thus the degree of "darkness" or its counterpart "luminosity" of a substance may be determined by placing it on the magnesia block corresponding to the slit of constant width, and then adjusting the width of the other slit in such a way that through the eye-piece of the instrument both halves of the field appear exactly alike, very much as is done in the well-known "half-shadow" polariscope. A special device is used to spread the light evenly over each half of the field. The scale reading gives the degree of darkness or luminosity of the sample under consideration. By the procedure just described the relative darkness of such substances can be determined which exhibit no specific color, but which merely reflect white light of varying intensity, as, for instance, the shades generally termed gray. If the instrument could do no more than this, its usefulness would indeed be very limited. However, a very simple device added to it converts it into one which allows us to read not only the depth, but also the quality of the color of any material. This is done by viewing the field through three glass plates showing the three primary colors, one red, one green and one blue. The field is in each case adjusted as before to the same depth of color in both halves, and the degree of darkness read on the scale. Thus we obtain the concentration of color for the three shades complementary to those of the three glasses, namely, green, red and yellow. By adding the three figures thus obtained in each reading, we arrive at the total concentration of the coloring matter, expressed in scale degrees of the instrument. The readings are very easily and quickly made, and an entire determination requires only about a couple of minutes. As has been mentioned above, certain solid substances like paper, cloth, etc., may be placed directly on the respective magnesia blocks, while powders, grained materials, fats, etc., are best placed in a tray of the same shape and height as the magnesia block and are put in its place. For reading liquids a special attachment must be used by which it is possible to have the light from the magnesia block pass through a column of liquid, while the possibility of any other light going through it is avoided. It stands to reason that in comparative work the height of the column of liquid must always be the same.

We were first led to an investigation of the merits of the tint-photometer by the great difficulties which we experienced in determining the decolorizing power on sugar products of the large number of vegetable carbons prepared in this laboratory.

Here we found, as we did later with the different products of the sugar factory, that the solutions decolorized with various carbons differed not only in depth of color, but also in tint. The tint-photometer helped to overcome this difficulty, and it is to be hoped that further researches may make it possible to refine the methods which we have used so far.

Since the readings obtained in degrees of the instrument bear no direct relation to the concentration of coloring matter, it was first necessary to standardize the instrument for the purpose of translating the readings into concentrations. To this end we prepared an aqueous solution containing 30 grams per liter of a dark final molasses, and made up a series of lower concentrations containing respectively 0.3 grams per liter, or 1 per cent of the original; 0.6 grams, or 2 per cent; and so forth for each percentage up to 20, then at intervals of 2 per cent each up to 30 per cent, and from then on at intervals of 5 per cent each up to 100, which latter is the concentration of the original solution. Thus we knew that the sample diluted to 50 per cent contained exactly one-half as much actual coloring matter as the original, the 20 per cent solution only one-fifth, and so forth. Each of these diluted solutions was then read in the tintometer and the readings recorded. Having obtained these figures, we could now, by comparing them with those obtained for the solutions decolorized with carbons, tell exactly what percentage of color each carbon removed from the solution, and further than that, could tell this percentage for each of the three colors. Diagrams were then drawn in which the readings of the instrument were plotted vertically, and the percentage concentrations horizontally. In this way we obtained four curves, one each for the three colors, and a fourth for the totals.

Unknown color concentrations could then be determined by reading equal volumes of these solutions in the instrument, and finding the corresponding concentrations from the diagrams. It is evident that these concentrations are only relative quantities, because we are unable as yet to determine the absolute percentage of total coloring matter even in our standard. This, however, does not greatly detract from the usefulness of the method for our purposes. We adopted the system of calling the color concentration of our standard molasses solution 100, and expressing all other concentrations on this basis.

In determining color in syrups, molasses and sugars, these products had to be first dissolved in water, in order to bring the concentration to within the limits of the solutions of known concentration. Calculations were greatly facilitated by dissolving quantities of syrup and molasses containing 10 and 5 grams respectively of total solids, and 25 grams of sugar, to 100 cc total volume. In all cases where decolorizing carbon was not used in the clarification, it was found necessary to remove finely suspended matter by heating with a little kieselguhr and filtering. This was needed especially in the case of raw juices. It must therefore be kept in mind that wherever the color of ''raw'' or ''mill'' juice is spoken of, we mean the liquid obtained after treating with kieselguhr. This latter substance in itself has a very slight decolorizing effect which is not sufficient, however, to affect the results.

The determinations of the two forms of iron and of the polyphenols were made in a similar way as those of total color, and in the case of the former two we could get really quantitative results. Series of solutions of known concentration of ferrous ammonium sulfate, and of ferric alum were made up, equal quantities of dilute hydrochloric acid and of solutions of potassium ferricyanide and potassium ferrocyanide respectively were added, and the solutions read in the tint-photometer. Diagrams were then drawn as before, and the unknown concentration of the constituents in our sugar products found by comparison with the readings obtained. In the polyphenol series the determmination can of necessity be only approximate. It was found, in the first place, that evidently certain polyphenols were eliminated during the process of manufacture, and others formed. In the second place, the exact nature of the polyphenols of the cane itself is not yet known, so that we had to have recourse to ordinary gallotannic acid to construct our concentration curves. The results are still comparative, but may deviate to some extent from the actual concentrations. As we had done before, solutions of known concentration of tannic acid were mixed with equal quantities of dilute ammonia and potassium ferricyanide solution, the readings taken, and plotted against the concentrations.

As was to be expected, the color readings of the products to be analyzed did not always agree in each color region with the

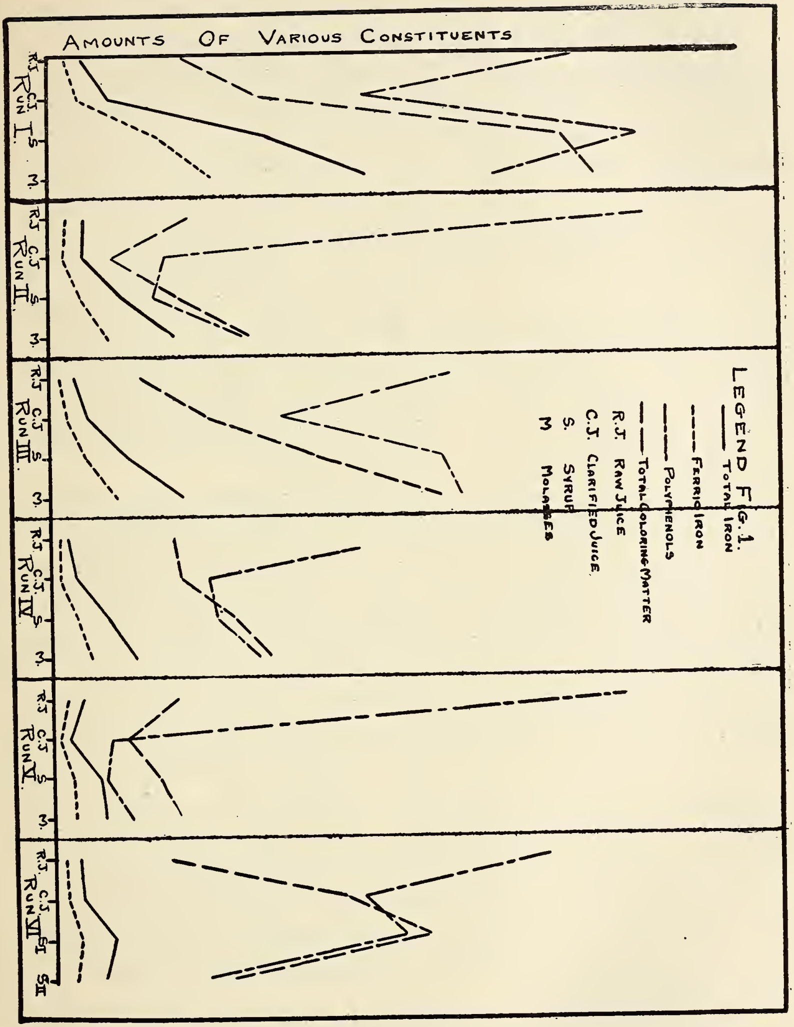
TABLE II.

PRODUCTS.	Color	Ferrous Iron	Ferric Iron	Total Iron	Polyphenols	Ferric Polyphenols
RUN 1.						
Raw Juice	16.5	11	9	20	325	72
Clarified Juice	26	20.5	16	36.5	195	128
Syrup	145	154	154	308	830	949
First Molasses	457	660	677	1337	1865	2130
First Sugar	52	94	110	204	260	297
RUN 2.						
Raw Juice	18	12	10	22	390	80
Clarified Juice	8	12.5	8	20.5	75	64
Syrup	45.5	66.5	54.5	121	181	207
First Molasses	188	314	269	583	900	1029
First Sugar	43	50	68	118	160	183
RUN 3.						
Raw Juice	13	11	6	17	295	48
Clarified Juice	22.5	15	11	26	170	88
Syrup	94	79	57.5	136.5	700	460
First Molasses	323	280	272	552	1700	1943
First Sugar	47	42	40	82	340	320
RUN 4.						
Raw Juice	14	5	3.5	8.5	220	29
Clarified Juice	15	8	4	12	90	32
Syrup	58	49	37.5	86.5	260	297
First Molasses	176	185	151	336	840	960
First Sugar	42	24	30	54	160	183
RUN 5.						
Raw Juice	13	8	7	15	300	56
Clarified Juice	7.5	4.5	3	7.5	30	24
Syrup	28.5	38	24	62	70	80
First Molasses	112	138	86	224	345	388
First Sugar	19	14	20	34	58	66
RUN 6.						
Raw Juice	12.5	8	4.5	12.5	260	36
Clarified Juice	32	9.5	5.5	15	170	44
Syrup, no carbon	109	49.5	33.5	83	515	268
Syrup, with carbon	37.5	29.5	19.5	49	155	156

readings obtained with those of known concentration, owing to differences in tint. But the totals gave a fair measure of the total depth of color, and besides, the readings taken in the region of medium wave lengths also showed good agreement. The figures given in the table above are based on the total readings. This table gives the total color, ferrous iron, ferric iron, total iron, polyphenols, and ferric-polyphenol compounds in all of the products made in the sugar house tests. As has been mentioned before, the figures for total color are only relative, and are based on the total coloring matter contained in the 3% molasses solution which is called 100. All other figures are in parts per million, those for the ferric-polyphenol compounds being calculated from the two constituents. The figures for the raw and clarified juices are averages of all the clarifiers in each run.

While the figures given in the table permit us to make some very interesting observations, a direct comparison of the products of each run with the corresponding ones of the other runs is not possible, because, although the juices were all of approximately the same density, the other products are so only in some cases, and then wholly by accident. In order to have a more secure basis on which such a comparison could be made, we have calculated all the figures on the basis of total non-sugars in each product (total solids less sum of sucrose and reducing sugars). The calculations could not be based on the total solids, because sucrose is removed when the first molasses is obtained. It is true that the total non-sugars also change somewhat in quantity, by clarification, scale formation, decomposition by high temperatures, etc., and any increase or decrease in constituents is partly due to a decrease or increase of the non-sugars. But these, nevertheless, afford the only basis on which such calculations can be based, and they should be fairly reliable for this purpose.

Since these important data can be shown much more readily and more convincingly by graphic methods, we have drawn some diagrams which will explain better than tables the points we wish to bring out. In Fig. I the lines consisting of long dashes give the total coloring matter—only in relative proportions, it must be remembered. All the other constituents are in parts per million parts of total non-sugars. The lines made of short dashes denote the ferric iron, the solid lines the total iron, and the lines consisting of alternate long and short dashes the polyphenols. In runs 1 to 5 the turning points in each line give the quantities of the various constituents in the four products, raw juice, clarified juice, syrup and molasses. In the sixth run we have raw juice, clarified juice, original syrup, not treated with carbon, and syrup treated with carbon.


Amounts Of Various Constituents
Run I.
Run II.
Run III.
Run IV.
Run V.
Run VI.
R.J
C.J
S
M
SI
SII
Legend Fig. 1.
Total Iron
Ferric Iron
Polyphenols
Total Coloring Matter
R.J. Raw Juice
C.J. Clarified Juice.
S. Syrup
M Molasses

Total color. The total color in the six raw juices does not vary a great deal, owing to the fact that in each run mixed varieties, of greatly varying color, were used.

Mixed juice samples taken daily showed somewhat greater variations. The effect of the anthocyanin in purple canes, as influenced by the pressure of the mills, is strikingly shown by some tests in which we compared juices from green cane and purple cane in samples taken from the different sets of rollers in the mill. These samples were collected directly from under the rollers of the first and third sets, and therefore had only minimum quantities of iron. A sample of mixed juice was also taken at the point of issue from the mill. There was only a very slight difference in the color of the first mill juice from green and purple cane, both showing about 10 units, the latter being a shade darker. But the third set of rollers, with its greater pressure, gave a juice with 27 color units from purple cane, and of only 21 from green cane. The mixed juice from purple cane showed 18.5 color units, and the green only 13.

The darkest average raw juice was obtained in run 2, for reasons which will become apparent in our further discussion.

Turning now to the color of the clarified juices, we note the remarkable fact that in all the runs made without carbon the color of the clarified juice is darker than that of the—boiled and filtered—mill juice from which it was made. In runs 1 and 3 the increase in color is quite considerable, less so in run 4 (phosphoric acid used), but much more marked in run 6, because here the sulfured juice was limed almost to neutrality. This, according to Schneller, produces a darker juice, because high acidity, as used in the other runs, decomposes the ferric polyphenol compounds with a corresponding lightening in the color.

In runs 2 and 5, on the other hand, where carbon was used, the total color is reduced very considerably, and not increased like in the other runs. This in itself constitutes a strong argument in favor of carbon.

From clarified juice to syrup, and from this to first molasses, the total color increases in all of the runs. This shows that new coloring matter is formed, because it increases faster than the total non-sugars. The rate of increase is quite considerable in runs 1, 3, and 6 (straight sulfitation, without carbon), less so in run 2 (straight sulfitation, with carbon), still less in run 4 (sulfitation-phosphatation, without carbon), and least in run 5 (sulfitation-phosphatation, with carbon). It appears therefore that from the standpoint of total color not only the use of carbon, but also that of phosphoric acid is commendable.

The lasting effect of the carbon treatment is shown very strikingly by the location of the curves in the diagram. The minima and maxima are within narrower limits, and both are located much lower in those runs where carbon was used than in the corresponding ones, where it was not.

In run 6 the total color of the carbon treated syrup is found to be very much lower than that of the untreated, again showing the favorable effect of the carbon.

Ferric iron is higher in the raw juice of the first two runs than in the others, evidently because the mill had not been used for almost a year and rust dissolves more readily in cane juice than does metallic iron. The high iron content in the second raw juice is partly due to stoppages on account of breakdowns in the mill. From the raw juice to the clarified juice the ferric iron increases in all those cases where no carbon was used, and decreases in those where it was employed. This is due to the fact that between the mill and the clarified juice tank the juice takes up more iron, from the machinery and the lime, than is removed by the ordinary process of clarification; but where carbon is employed, so much iron is actually removed, in spite of all the juice being passed through the filter press with its large iron surfaces, that the clarified juice really contains less iron than the mill juice.

From the clarified juice to the syrup and to the molasses the iron increases rapidly, for reasons already stated. In fact, it rises much more rapidly than the concentration. But even in the molasses of those runs where carbon was used in clarification, the iron is still quite a bit lower than in the others.

A comparison of the entire ferric iron curve in run 1 with that of the other runs brings out a very interesting fact. Harloff and Schmidt[7] state that ''at the commencement of the campaign the first sugars will assume a gray or yellow tint which, according to researches conducted with this object in view, owes its origin to suspended impurities in the juice derived from insufficiently cleaned apparatus or pipes, and not, as was formerly supposed, to some insoluble coloring substance or iron.'' A comparison of the ferric iron curve in run 1 with that of the others readily shows that the conclusion does not hold true in all cases. The ferric iron in run 1, first molasses, is about two and a half times as high as that in run 3, which is the next highest.

In the treatment of syrup with carbon in run 6, the ferric iron is also reduced to some extent, just as happens in the juice treatment, but the decrease is not nearly as pronounced as the reduction in the total color.

Total iron. This is of less importance than the ferric iron alone, for reasons already explained. We might say that the ferrous iron has to be considered only inasmuch as it gives us a measure of the potential quantity of ferric iron which would be present after oxidation of all the ferrous iron to the ferric form. In comparing the total iron curves, about the same observations are made as with the ferric iron curves.

Polyphenols. The curves obtained for these compounds are of particular interest. It is readily seen that the polyphenol content is highest in the raw juice of runs 2 and 5, that is before any treatment whatever. These are exactly the two runs in which we ground a large number of small samples of seedling varieties, many of which were quite poor, and their polyphenol content was so pronounced that it affected the average. These findings confirm again the observations of Browne and Schneller, to the effect that unripe cane is higher in polyphenols than ripe cane.

In the process of clarification, the polyphenols are invariably reduced. But here again the use of carbon shows its great efficacy. In spite of the fact that the initial polyphenol content in the raw juices of the carbon runs 2 and 5 happened to be higher than that in any other run, the polyphenols in the clarified juices of the same runs 2 and 5 are ever so much lower than in the others.

The changes in polyphenols from the clarified juice to the syrup are likewise very interesting. It will be noted that in all cases where no carbon was used there is an increase from clarified juice to syrup, but a decrease in those runs where carbon was added to the other clarifying agents. This is probably partly due to the fact that certain compounds (saccharetin?) which give rise to polyphenols upon heating are removed by the carbon, so that the effect which they would otherwise exert is also prevented. But this would not explain an actual decrease between clarified juice and syrup. It would seem that some polyphenols are decomposed by heat and others are formed. A rise or fall would then occur according to the preponderance of one reaction over the other. As a rule the formation of new polyphenol compounds (pyrocatechin?) seems to exceed the destruction of others. In all of the molasses, excepting that of run 1, the polyphenol content is higher than in the syrup, owing evidently to the formation of new ones.

In the syrup of run 6, the carbon caused a very large reduction in the polyphenols, showing again the efficiency of the carbon in this respect.

The diagrams clearly demonstrate that the color does not change parallel with the polyphenols themselves, while there was indeed found a certain parallelism between the color and the ferric and total iron. The significance of this will at once become apparent in the following discussion.

Ferric polyphenol compounds. Although the clarified juice in run 1 has less polyphenols than the raw juice, the iron is so much higher that it raises the ferric polyphenol compounds, and also the total color, above those of the raw juice. The same is true of runs 3, 4, and 6, all of which were without carbon. But in runs 2 and 5, where carbon was used, the ferric polyphenol compounds in the clarified juice are less than in the raw juice, as are also the polyphenols, ferric and total iron, and total color.

The importance of the combined effect of iron and polyphenols is strikingly shown by the curves in Fig. 2, where the relative changes in the total color are given side by side with the changes in the ferric polyphenol compounds. It is seen, in the first place, that the two curves in each case where the final acidities were high (runs 1 to 5) are quite close together, or in other words that the color caused by the ferric polyphenol compounds is in a nearly constant proportion to the total color, excepting in run 6, where the low acidity explains the discrepancy. Moreover, there is seen a remarkable constancy in the direction of the changes of ferric polyphenol compounds and of total color. In 17 cases out of a total of 18 changes, a decrease in one means a decrease in the other, and an increase in one is accompanied by an increase in the other. So far we have not been

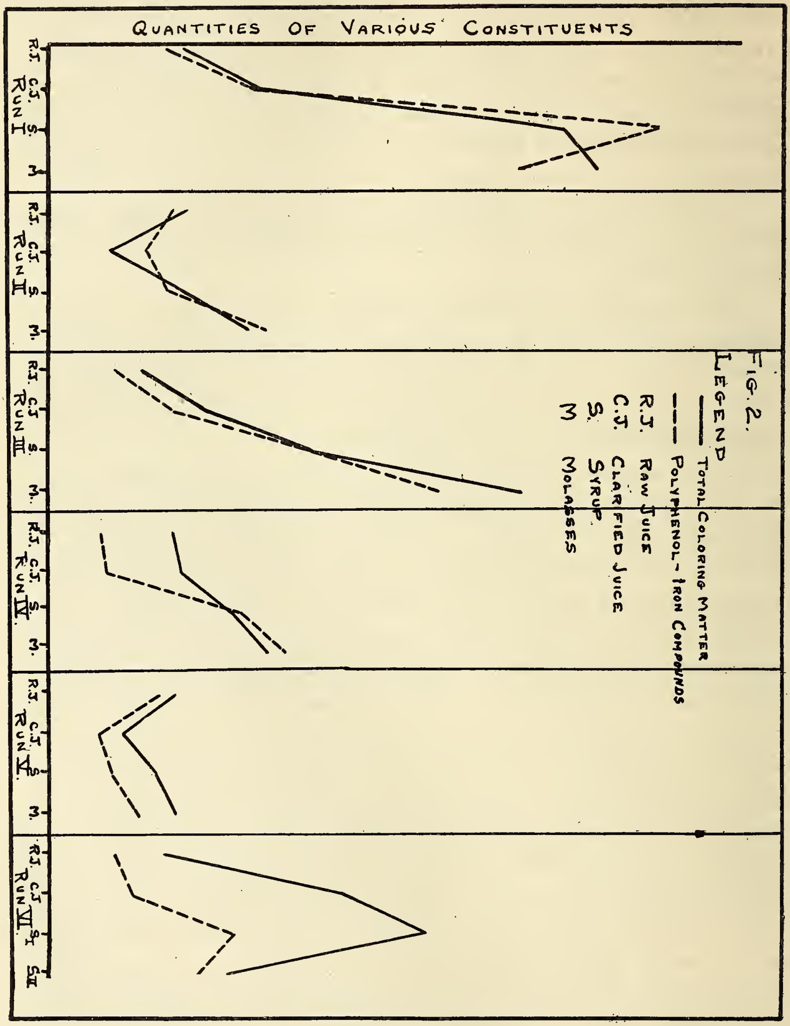


Fig. 2.
Quantities of Various Constituents
Legend
Total Coloring Matter
Polyphenol - Iron Compounds
R.J. Raw Juice
C.J. Clarified Juice
S. Syrup
M Molasses
R.J.
C.J.
S.
M.
Run I
Run II
Run III
Run IV
Run V
Run VI
S.I
S.II

able to determine what percentage of the total color is due to the ferric polyphenol compounds, but expect to solve this question also as soon as we have a sufficient quantity of cane polyphenol on hand. For the present we must content ourselves with knowing that the total color generally increases and decreases with the ferric polyphenol compounds.

The diagrams in Fig. 2 again show forcibly the effect of the carbon, inasmuch as there is not only a reduction in total color and in ferric polyphenol compounds where it was used, and an increase where it was not, but the total range of change in all the products is within much smaller limits, and of smaller magnitude throughout in runs 2 and 5 than in the corresponding ones 3 and 4.

Sugars. The figures for the sugars, shown in Table 1, generally lie between those for the clarified juice and those for the syrup of the same run. The total color, and, as was to be expected, also the ferric polyphenol compounds, are higher in the sugars from runs 1 and 3 (no carbon) than in run 2 (carbon), and higher in run 4 (no carbon) than in run 5 (carbon), although the proportions of ferric and total iron may not go parallel at all with them. They happen to be in the same direction as the polyphenols themselves, because in the sugars, like in the molasses and some of the syrups, the ferric iron is in excess of the polyphenols with which it can combine.

All these relations are shown even better in Fig. 3 than in Table 1. The five curves in the diagram give the relative quantities of total color, ferric iron, total iron, polyphenols, and ferric polyphenol compounds for all the five sugars made, based on the quantities of each found in sugar from run 1 (100%). It is seen that the total color is less in sugar 2 than in 1 and 3, due to the carbon. In number 4 it is less than in number 3, owing to the better effect of clarification with the use of phosphoric acid; in number 5 it is still lower than in 4, owing to the effect of the carbon in addition to the reagents used in run 4.

With both the ferric and the total iron the curves have an entirely different shape than the color curve, both ingredients decreasing steadily from one sugar to the next.

The polyphenols, however, change qualitatively in the same way as the total color, but from a quantitative standpoint there is but slight parallelism.

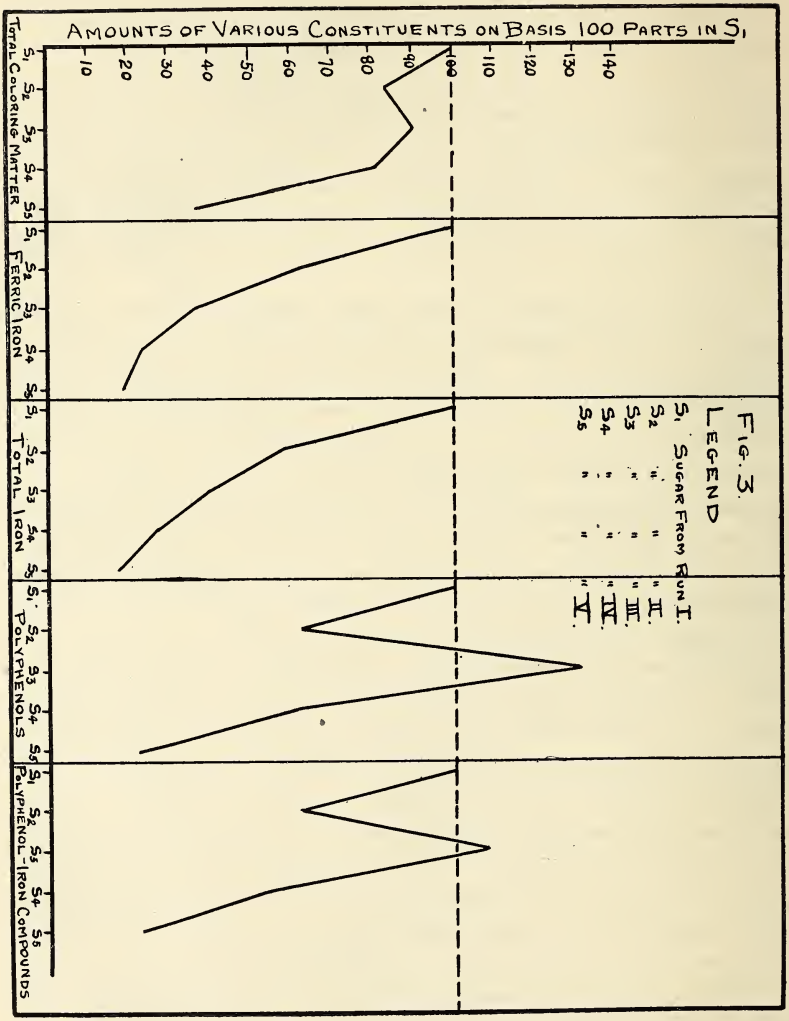


Fig. 3.

Amounts of Various Constituents on Basis 100 Parts in S_1
10
20
30
40
50
60
70
80
90
100
110
120
130
140
S_1 S_2 S_3 S_4 S_5
Total Coloring Matter
Ferric Iron
Total Iron
Polyphenols
Polyphenol-Iron Compounds
Legend
S_1 Sugar From Run I.
S_2 " " " II.
S_3 " " " III.
S_4 " " " IV.
S_5 " " " V.

The ferric polyphenol compounds also change qualitatively like the total color and the polyphenols, and from a quantitative standpoint the ferric polyphenol compound curve agrees most closely of all with the total color curve. This proves again the large part played by these compounds in the total color of the products, and confirms our observation with the other products.

The results obtained on the sugars, as shown in Table 1, also corroborate an observation made by Prinsen Geerligs[24], who found that the color of the sugars is darker than it should be, if it were all contained in the surrounding molasses film. This holds true for our sugars also, but to a smaller extent.

EFFECT OF DIFFERENT METHODS OF CLARIFICATION, AND OF DECOLORIZING CARBON IN ADDITION, ON THE YIELD OF FIRST SUGAR.

So far we have dealt only with the color of the products, and certain constituents of it. These have proven the efficiency of decolorizing carbons from this point of view; but we are naturally just as interested in the yield of the sugars obtained by the use of carbon, as compared to that made without its use.

This question may be decided from the results given in Table 1 on page 14.

In comparing runs 2 (with carbon) and 3 (without carbon) we notice first that the purity of the raw juice in run 2 was 3.6 points lower than that of the raw juice in the run to be compared with it. But in the clarified juice this difference has narrowed down to 2.8 points, on account of the fact that the clarification, plus carbon treatment, gave an increase in purity of 1.0, while clarification without carbon gave one of only 0.2. In the syrups the difference in purities became still smaller, amounting to only 2.3 points. The same observations are made in comparing run 2 with run 1, which was made in the same way as run 3.

Comparing run 4 (without carbon) and run 5 (with carbon), we again find a difference in purity of raw juice of 0.9, which narrows down to 0.3 in the clarified juices, and to nothing in the syrups. The results in both sets therefore agree well with those obtained in the 1917 tests.

The glucose ratio was not affected to any extent by the carbon treatment, which likewise confirms previous results.

The drop in purity from syrup to first molasses was 21.8

points in run 2, 20.5 points in run 3, and 17.3 in run 1, a difference of 1.3 and 4.5 points in favor of the carbon run. In runs 4 and 5 the differences were smaller, only 0.2 points in favor of the carbon run, undoubtedly because the purity of the two syrups was the same. Mr. Taggart noticed again, as he had in 1917, that the carbon treated products boiled more freely than those not so treated. This is also in line with the observations of Peck and Adams, already mentioned. The yield of sucrose in the form of first sugar, calculated on 100 parts sucrose in the syrup, was 57.3 in run 1, 62.6 in run 2, 62.1 in run 3, 58.6 in run 4, and 59.0 in run 5. Slightly better yields were therefore obtained from each of the carbon treated products than from the untreated, in spite of the fact that the raw juices were poorer in each case. The glucose ratio also rose less, from syrup to molasses, in run 2 than in run 3, and less in 5 than in 4. This fact shows again the better quality of the carbon treated products.

The quality of the raw sugar, made in each case without the use of wash water in the centrifugal, was better in run 5 than in run 4, the former having 95.1% sucrose, and the latter only 93.7. In runs 2 and 3 the result was different, owing no doubt to the fact that the purity of the syrup in run 2 was still considerably lower than in run 3, and also that the massecuite in run 2 was boiled much stiffer. For this reason the massecuite of run 2 did not purge as well as that of run 3. Sugar No. 2 has 90.7% sucrose, and sugar No. 3, 93.7%. It is certain that under more equal conditions the sugar of run 2 would have been at least as high in sucrose as that of run 3.

CONCLUSIONS.

The results obtained in our experiments show that the combination of ferric iron and polyphenols indeed plays a very important part in the color of our sugar house products. If it is desired to make a light colored sugar, it is necessary to keep both iron and polyphenols at a minimum. The former condition can best be accomplished in actual practice by working as rapidly as possible, and by not leaving any of the products in contact with iron surfaces any longer than is absolutely necessary. As Schneller has pointed out before, iron machinery might to advantage be largely replaced by copper, especially in those countries where the sugar factory is in use all the year around. In certain cases it might pay to make raw sugar during the campaign, and afterwards refine this by the use of declorizing carbon in connection with a copper pan. It would also be advisable to paint all iron machinery wherever possible with iron-free paint which should be quite resistant to the effect of acids, alkalis and heat. When boiling out with caustic soda, a solution of the desired strength should first be prepared so that the iron or painted surfaces do not come in direct contact with solid caustic or strong lye. Iron in the products may also be largely reduced by the use of decolorizing carbon.

The polyphenols are removed only to a slight extent by the ordinary methods of clarification used in white sugar manufacture in Louisiana. Decolorizing carbons are, however, very effective in taking out polyphenols, and also other compounds which in the course of manufacture give rise to new polyphenol compounds. If a sufficient quantity of an active carbon be used, the polyphenols, the iron combined with them, and also the mother substances of polyphenols may be removed practically completely. The question as to how far one may go economically in this respect, will of necessity have to be determined by actual factory tests on a normal scale.

Our tests have further demonstrated that the yield of sucrose in the form of first sugar is not smaller, but even slightly better where carbon is used than where it is not. But of even greater importance is the fact that the use of carbon produces a first molasses of much lighter color. The significance of this has been pointed out by Harloff and Schmidt[7]. It would naturally be expected that a molasses of light color will give a light colored sugar as well, and that therefore a larger quantity of high grade sugar could be made by boiling back such a light colored molasses. Experiments on this particular point are contemplated for the coming grinding season at our sugar house.

It is evident that the results obtained at this station warrant tests on a larger scale on the part of our sugar planters, and the hope is again expressed that at least some of them will be sufficiently interested to undertake this work at the earliest possible moment. The fact that the assistant director of this station has finally succeeded in securing the free use of decolorizing carbon from rice hulls for the public, should be a further incentive in this direction.

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The Deterioration of Cane Sugar by Fungi

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The Deterioration of Cane Sugar by Fungi*

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INTRODUCTION.

The deterioration of food products has assumed an added significance during the war which it seems likely to maintain. This applies to sugar for the familiar reasons as well as the complicating problems of equalization, distribution and reserve. The problem of sugar deterioration has long been the subject of investigation and since the original observations of Payen[33] and Dubrunfaut[11], it has been considered to be of a microbiological nature. The contributions in this field have been comprehensively reviewed by Owen[29 30 31 32] and others, so that it is unnecessary at this point to do more than note that the emphasis in such research has been, to a great degree, on the bacterial flora involved, as evidenced by the valuable contributions of Lewton-Brain and Deerr[5], Deer and Norris[9], Dodson[10], Greig-Smith[19], Fermi and Montesano[16] and Greig-Smith and Steel[21]. More recently, however, there has been an increasing tendency to regard the fungi (or molds, as they are more commonly termed) as playing an important rôle in the inversion of sucrose which occurs in manufactured cane sugar.

Since comparatively little work has been done with fungi as a starting point, it was considered advisable to undertake a survey of the fungus flora of the different types of cane sugar and then to determine the power which the most important of these might have in causing deterioration. Subsequently the presence of these organisms in the sugar mill was studied with a view towards perfecting methods for the elimination of the dangerous invaders. It is not to be assumed that the experimental data and their interpretation set forth in the following pages are complete, but, rather, that they represent a report of progress which may prove of interest and possibly suggest methods for the conservation of that food product in which there is universal concern and which is continually subjected to deterioration by the activity of microorganisms.

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HISTORICAL.

Payen*[33] as early as 1851 noted a red coloration in sugar. Upon isolating the organism responsible he found that the spores were 1-2 microns in diameter. In a later communication he describes sugar which had irregular ridges on the crystals and cavities. On these were found filaments and their ramifications which were different from those of the previous organism described.

Shorey[40] found *Penicillium glaucum* in deteriorating Hawaiian sugars and regarded this organism as the factor responsible for inversion. This evidence, he claims, proves that the assertion "that all sugars which deteriorate do so because too moist or not alkaline enough" is unwarranted. The only place in the sugar-making process where the spores of fungi could gain access to the sugar is in the centrifugal and he finds a correlation between dusty districts and deteriorating sugars. Sterilization by dry steam in the centrifugal is recommended as protection against infection.

Kamerling[22], whose work is favorably discussed by Geerligs[17], found in dried sugar a fungus flora chiefly related to *Penicillium*. Over twenty varieties were observed and not less than nineteen belonged to this group. The author claims that during the course of deterioration of raw sugar the first attack is made by fungi.

Greig-Smith[20] advises the same procedure as Shorey[40] to guard against a bacterial infection. The former has isolated *Aspergillus glaucus* from small-grained, moist refined sugar, but has not considered its presence to be of as much significance as the potato group of bacilli, upon which he has worked so extensively.

Schöne[36 37 38] emphasized the importance of molds and torulæ in inversion and noted the presence of *Penicillia*, especially *P. glaucum* and *Mucor*. He found that considerable acidity was developed in sugar solutions inoculated with pure cultures of some of these organisms. In another connection he notes the isolation of *Penicillium* and *Rhizopus*.

Scott[39] observed the fungi on the surface of sugar liquors, noting *Penicillia* and *Aspergilli*. He found that sugars were attacked by these organisms in the following descending order: Low Brazilian, Peruvian, Jamaican and Javanese. The inverting power of the fungi was noted and precautions advised against infection.

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* The authors are indebted to Dr. C. A. Browne for this abstract.

Amons[2] isolated by plating on synthetic agar 4 *Aspergilli,* 2 *Penicillia* and a *Rhizopus.* Mycelial threads observed in moist sugars were identified as indicating the presence of *P. glaucum* in an active form. The deterioration of sugar by this fungus was demonstrated by inoculating sugar (sterilized with absolute alcohol) with spores and comparing the absorption of moisture (from a saturated sterile sugar solution under a bell jar) with a sterile check. It was found that the sterile sugar absorbed no moisture after thirty-three days, while the inoculated sugars absorbed a considerable amount of water. This is interpreted as signifying an appreciable fungus growth which inverted the sucrose, the hygroscopic reducing sugars being responsible for this absorption of moisture. It would have added to the value of this experiment had the chemical analyses for sucrose, reducing sugars, etc., been recorded at the beginning and end of the incubation period. Amons believes with Browne[7] and Noel Deerr that no single organism can be made universally responsible for the deterioration of sugar. Our evidence tends to corroborate this view.

Browne [6] [7] in the course of his contributions to the sugar industry has included a great number of chemical observations concerning sugar deterioration. He has discussed the influence of temperature upon deterioration, deterioration without loss in polarization, the distribution of moisture, sweating, abnormal fermentations, the limit of deterioration, and has recorded practical deductions.

In his mycological observations Browne isolated and studied the inverting power of torulæ and two *Monilia* (named "nigra" and "fusca" respectively). The presence of fungi such as *Penicillia* were noted and their possible importance emphasized. Some interesting points are made with regard to sources of infection and consequent prevention of deterioration of raw cane sugars.

Owen [29] [30] [31] [32] after an exhaustive survey of the activities of bacteria and torulæ investigated the role played by molds in sugar deterioration. From a large variety of Louisiana sugars he isolated a number of *Aspergilli* and studied their activity in pure culture in sugar solutions of high concentration.

In an interesting group of experiments concerned with the conditions limiting the respective activities of the three groups of microorganisms in sugars, Owen[32] found that a fungus inoculation caused marked deterioration in a 69° Brix solution, which proved to be of too great a density for the activity of either bacteria or torulæ, the latter two groups inverting sucrose at 52° and 64° Brix respectively as the upper limits. He regards the fungi as constituting the most dangerous group of microorganisms in sugar because of their strong inverting power, their ability to exercise this power in highly concentrated solutions of sucrose of varying reaction, and also on account of their ability to develop on media which are very deficient in nutrients.

Amons[3] and Edson[13] have likewise indicated the activity of fungi in syrups.

Blake[4] has recently made a contribution to the deterioration of raw sugar, as has Prinsen Geerligs[18]. They are especially interesting in that they represent observations made from the factory rather than the laboratory standpoint, and will doubtless result in more enlightened practices because of the economic considerations involved.

PART I.

THE OCCURRENCE OF FUNGI IN CANE SUGAR.

It is evident from this review of previous investigations that there is an informing body of data which makes it imperative to subject the fungi to closer scrutiny with regard to their ability to deteriorate manufactured cane sugar.* With this end in view, a comprehensive survey of the fungus flora of cane sugar was undertaken. The samples under consideration included a variety of types of sugar from different sources and represented a considerable range in composition, age and keeping quality. For example, all sugars numbered up to 50 are samples which have been kept in tightly covered Mason jars for about two years. Numbers above 100 indicate sugars collected during the past year, many of these samples being employed in the laboratory within a very short time after manufacture, in some cases this being only a matter of a few hours. Samples of the principal types of sugar on the market are designated as follows: Plantation granulated, which is a dry white granulated sugar manufactured on a large scale in Louisiana and is sold without further refinement; ''Plantation granulated undried,'' similar to the above but possessing more moisture; Yellow clarified, which is a still lower grade of the same process; Standard granulated, which is the dry refined sugar; Cuban raw, the standard raw sugar imported from Cuba; 96°, which takes its name from its polarization and implies a raw sugar; 96° washed, which means a low grade sugar ''washed up to'' a higher polarization; and, finally, Second, which is the term given to the raw sugar obtained from the extraction of first molasses.

The above mentioned types are representative not only of local but national sugar standards.

The isolation of fungi from these representative sugars was accomplished by the plating method commonly employed in bacteriological technique. In order to have a dilution water of as nearly the same approximate composition as the original source from which the isolations were being made, a 17° Brix solution made up with Plantation granulated sugar and sterilized in the autoclave at 15 pounds' pressure for 15 minutes was employed, thereby reducing any danger from plasmolysis, etc.[32] The method of plating was to dilute 100 gm. of each sugar in 100 cc. of dilution water, dissolve and shake thoroughly while removing 1 cc. with a sterile pipette to a sterilized Petri dish. Eight cc. of agar medium cooled to 35°-40°C. were then added and the plates incubated at 28°-30°C. for one week, counts being made after 3, 5 and 7 days respectively. With media having 50 per cent of sucrose the plates were examined again after 10 days. It may be observed, parenthetically, that we have found it desirable to pour media as cool as possible (even permitting a slight hardening in the bottom of the tube) to avoid any injurious heating of sensitive spores.

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* Some of the results included in this bulletin have appeared in our preliminary communications [24] [25] [26].

Media were employed which were considered to be adapted to a wide range of fungus development (sucrose being substituted for the sugar mentioned in the original formula), as well as some having a high sucrose content, and therefore more closely approximating the original habitat of the organisms isolated. All media were made up to a reaction of +1 by the usual titration method. As a rule, all media high in sucrose content were sterilized intermittently at streaming steam for 3 successive days, but towards the conclusion of the work a single sterilization in the autoclave at 15 pounds' pressure for 15 minutes was considered more satisfactory. This is in line with the results obtained by Mudge[28].

Under the high temperature and humidity conditions obtaining in our vicinity and because of the high concentration of the original product, it was desirable to use a stiff agar (20 gm. "Difco" per litre, all media being made in a double boiler) and it was found to be advantageous to plunge the media into cold water upon removal from the autoclave.

The synthetic fungi media in general use, Czapek's, Cook's II and Raisin agar, were compared with the media developed and recommended for sugar investigations by Amons[2], Browne[7] (using granulated sugar, instead of raw sugar, as suggested by this author) and Smith. In the latter case, Owen's modification of using 50 per cent sucrose was also employed. Hansen's yeast medium was included for comparison, as well as a modification of Czapek's agar by the authors. This modification consisted of increasing the sucrose content from 30 to 50 gm. per litre and substituting 5 gm. of peptone and 1 gm. of ammonium nitrate for 2 gm. sodium nitrate in the original formula. This resulted in a more rapid colony development, which proved to be a time-saving device of some value. The composition of the media employed was as follows, using 20 gm. of agar in 1000 cc. of water:

NAME	Sucrose, gm.	Peptone, gm.	K_2HPO_4, gm.	KC1, gm.	$MgSO_4$, gm.	$FeSO_4$, gm.	Additional gm.
Amons	100		1.0		0.5		2.0 KNO_3
Cook II	20	10	.25		.25		
Czapek	30		1.0	.5	.5	.01	2.0 $NaNO_3$
Hansen	100	10	3.0		2.0		
Kopeloff	50	5.0	1.0	.5	.25	.01	1.0 NH_4NO_3
Smith 10%	100	1.0		5.0			2.0 Na_3PO_4

The granulated sugar medium (identical with Browne's formula for raw sugar) was made by filtering a 30 per cent sucrose solution and diluting to 20° Brix. The Raisin agar was made by cooking 375 gm. of raisins for 1 hour at 80°C. and filtering, adding 2 gm. of NH_4C1, and making up to one litre, with a reaction of +2.0. Smith's 50 per cent sucrose agar (Owen's modification) was identical with the formula given for the 10 per cent agar except that the sucrose content was increased from 100 to 500 gm.

TABLE I.

ISOLATION OF FUNGI FROM SUGAR.

Key:—Asp. f.—Aspergillus flavus. Asp. n.—Aspergillus niger.
Asp. nid.—Aspergillus nidulans. Asp. r.—Aspergillus repens.
Bl. Asp.—Blue Aspergillus. Clad.—Cladosporium.
Cit. I, II, III—Citromyces I, II, III.
Orange—Sterile Orange Fungus. Pen.—Penicillium sp.
Pen. div.—Penicillium divarticatum.
Pen. exp.—Penicillium expansum. Pen. lut.—Penicillium Luteum.
Pen. ros.—Penicillium roseum. Syn.—Syncephalastrum.
Trich.—Trichoderma. I, II, III, IV—Unknown Sterile I, II, III, IV.
F?—Unknown Fungus.

Type	No.	Amons	Cook	Czapek	Gran. Sugar	Hansen	Kopeloff	Raisin	Smith 10%	Smith 50%
P. Gr.	1	0	Asp. n. Clad. Orange	Asp. n. Bl. Asp. Orange Cit. I Clad.	Clad.	0	Asp. n. II Bl. Asp.	Asp. n. Bl. Asp Cit. I Orange	Asp. n.	Asp. n. Clad. II Bl. Asp.
96°	2	Asp. n. Bl. Asp.	Asp. n. II	Asp n. Bl Asp.	II	Asp. n II	Asp. n.	Asp. n.	O	Asp. n. Bl. Asp.
P. Gr.	3	Bl. Asp. Orange	Bl. Asp.	Asp. n. Bl. Asp. Orange II Clad.	Bl. Asp.	Asp. n. Bl. Asp.	Asp. n. Bl. Asp. III	Asp. n. Clad. Bl. Asp. Pen. lut.	Bl. Asp.	Asp. n. Bl. Asp. Pen. div.
96°W	7	Asp. n.	Asp. n.	Asp. n. Pen. div. IV Asp. fl.	Asp. n.	Asp. n. Bl. Asp.	Bl. Asp. II	Asp. n.	Asp. n.	Asp. n.
96°W	8	Asp. n. F?	Bl. Asp. Asp. fl.	Asp. n. Clad. Pen. div. II	O	Asp. n.	Bl. Asp.	Asp. n. Clad. Bl. Asp.	F?	
96°W	9	O	Bl. Asp.	Asp. n. Clad. Pen. exp. II	O	Asp. n. Bl. Asp.	Bl. Asp.	Bl. Asp. Clad.	Clad.	Bl. Asp. Asp. fl.

2nd	11	Asp. n. Bl. Asp. II	Asp. n.	Asp. n. Clad. II F?	F?	Asp. n. Orange III	Asp. n. III	Asp. n. Asp. fl.	Asp. n.	Asp. n.
96°W	12	Bl. Asp. F?	Asp. n. Bl. Asp.	Asp. n. Clad. IV Bl. Asp	O	Asp. n. Bl. Asp.	IV	Asp. n. Clad.	Clad. Pen. exp.	Bl. Asp. Clad. Cit. III
96°W	13	Clad. Bl. Asp.	Bl. Asp.	Asp. n. Bl. Asp. Clad. Pen. exp. Asp. fl.	O	Asp. n.	Asp. n. Asp. fl.	Asp. n.	Asp. n. Clad.	Asp. n. Clad.
96°W	14	Bl. Asp.	Bl. Asp.	Asp. n. Bl. Asp. Pen. exp.	O	Asp. n. Bl. Asp.	Bl. Asp.		Asp. n. Clad. Bl. Asp.	Bl. Asp.
P. Gr.	15	O	Asp. fl.	Asp. n II Bl. Asp.	O	Asp. n. Bl. Asp. IV Orange Cit. III	Asp. n. Bl. Asp. II III	Asp. n. Bl. Asp.	Clad F?	Asp. n Bl. Asp.
P. Gr.	16	Asp. n.	Asp. n. Clad. Bl. Asp. Orange III	Asp. n. Clad. Bl. Asp. II	Bl Asp.	II	Clad. III	Asp. n. Bl. Asp	Asp. n. Bl. Asp.	Asp. n. Bl. Asp.
P. Gr.	17	Bl. Asp. Orange	Bl. Asp. Orange	Asp. n. Bl. Asp.	Syn.	O	IV	Asp. n. Clad. Bl. Asp.	Bl. Asp. Syn.	Bl. Asp.
2nd	18	Clad.	Clad.	Clad. Asp. fl. II, IV.	O	Asp. n Bl. Asp.	O	Asp. n. Clad.	Asp. n.	
96°W	19	O	Bl. Asp.	Asp. n. Clad. Bl. Asp. IV	Clad.	Asp. n.	Asp. n. II	Asp. n.	Bl. Asp. Clad.	Asp. n.

TABLE I—Continued.

ISOLATION OF FUNGI FROM SUGAR.

Type	No.	Amons	Cook	Czapek	Gran. Sugar	Hansen	Kopeloff	Raisin	Smith 10%	Smith 50%
2nd	20	Asp. n. Clad. Bl. Asp.	Asp. n. Syn. III	Asp. n. Clad. Bl. Asp. Pen. Lut. Orange III	Asp. n. Bl. Asp. IV	Asp. n.	Asp. n. Clad. Orange IV Cit. III	II Clad.	Asp. n. Bl. Asp. Clad. Pen. exp. Orange IV	Asp. n. Bl. Asp. Clad. II Trich.
96°	21	Asp. n. Clad. Bl. Asp. II	Asp. n. Orange	Asp. n. Clad. IV	Asp. n. Bl. Asp. IV Orange Asp. fl.	Asp. n.	Asp. n. Bl. Asp. Asp. fl.	Cit. I	Asp. n. Bl. Asp. Asp. fl.	Asp. n.
P. Gr. und.	22	Asp. n.	Clad. Bl. Asp. Orange	Asp. n. Clad. IV F?	Asp. n.	O	Asp. n. Bl. Asp.	Clad.	O	Asp. n. Asp. fl.
96°	23	Pen. exp. Syn.	Syn. Asp. n. Bl. Asp.	Asp. n. Bl. Asp. Clad. Syn.	Asp. n.	Asp. n.	Asp. n. Pen. exp.	Asp. n. Cit. I	O	Asp. n. Clad. Asp. fl.
2nd	24	Asp. n. Asp. fl.	Asp. n. III	Asp. n. Clad. Asp. fl. Orange Cit. III.	Asp. n. Clad.	Asp. n.	Asp. n. Bl. Asp. Asp. fl. III	Asp. n. Asp. fl.	Bl. Asp. III	Asp. n. Asp. fl.
P. Gr. und.	25	Asp. n.	Bl. Asp. Asp. fl.	Asp. n. Bl. Asp. Clad. II Asp. fl. Pen. exp.	O	II	Bl. Asp. Clad. II	Asp. n. Clad. Bl. Asp.	Bl. Asp. Pen. exp.	Bl. Asp.
96°	26	Asp. n.	Asp. n.	Asp. n. Pen. exp. Clad. II, IV	Asp. n. Bl. Asp.	Asp. n. Bl. Asp. III	Asp. n. Bl. Asp. Asp. fl.	Asp. n. Clad. Bl. Asp.	Bl. Asp.	Asp. n.

96°	27	O	Asp. n. Bl. Asp.	Asp. n. Bl. Asp. Clad.	Clad.	Asp. n.	Asp. n. Clad. Orange	Asp. n. Bl. Asp.	Clad.	Bl. Asp. III
P. Gr. und.	28	Bl. Asp.	Asp. n. Bl. Asp.	Asp. n. Clad. Pen. lut. Bl. Asp. II	Bl. Asp.	Asp. n.	Asp. n. Clad. Orange	Clad.	Bl. Asp.	Asp. n. Bl. Asp. II, III
P. Gr.	29	Bl. Asp.	Bl. Asp. Asp. fl.	Asp. n. Bl. Asp. Pen. exp. II	Clad.	Clad.	Bl. Asp.	Asp. n. Clad.	O	Clad. III
2nd	30	Asp. n. Bl. Asp. Clad.	Bl. Asp. Cit. III	Asp. n. Clad. II	Bl. Asp.	Asp. n. Clad.	Asp. n. Bl. Asp.	Asp. n.	Asp. n.	O
96°W	31	Asp. n. Clad. Bl. Asp. III	Clad.	Asp. n. Clad. Bl. Asp. Pen. exp. II	O	II	O	Asp. n.	Asp. n.	Asp. n. Clad.
P. Gr.	32	Asp. n.	Asp. n. II	Asp. n. Clad. Pen. exp. II	Asp. n.	Asp. n. Bl. Asp.	Asp. n. II	Asp. n. Pen. lut.	Asp. n. Bl. Asp. Clad.	Asp. n. Bl. Asp. Clad. Orange III
96°	33	Asp. n. II Pen. lut.	Asp. n. Bl. Asp. Clad. Pen. exp.	Asp. n. Bl. Asp. Pen. exp. II, III Orange	Asp. n. Asp. fl.	Asp. n. Clad. Syn. Orange	Asp. n. Bl. Asp. Clad. Asp. fl. Cit. III	O	Asp. n. Bl. Asp. Asp. fl. Orange III	Asp. n. III
96°W	34	Asp. n. Clad. II	Asp. n. Bl. Asp. II	Asp. n. Bl. Asp. Clad. Pen. exp.	II	O	Asp. n. Clad. Orange III	Asp. n.	Bl. Asp. Clad. Syn.	Asp. n
P. Gr.	35	Asp. n. Clad. Bl. Asp. III	Pen. exp. III	Asp. n. Pen. lut. Clad. Syn. Orange Bl. Asp.	O	O	Asp. n.	Asp. n. Cit. I	Bl. Asp.	Asp. n.

TABLE I—Continued.

ISOLATION OF FUNGI FROM SUGAR.

Type	No.	Amons	Cook	Czapek	Gran. Sugar	Hansen	Kopeloff	Raisin	Smith 10%	Smith 50%
2nd	36	Bl. Asp.	Asp. n. Bl. Asp.	Asp. n. Asp. fl. Clad. Cit. I II	F?	O	Asp. n. Bl. Asp.	Asp. n. Bl. Asp.	F? IV	Asp. n. Bl. Asp. Clad. III
2nd	37	Asp. n. Bl. Asp.	Asp. n. Bl. Asp. Clad.	Asp. n. Bl. Asp. II Clad. Asp. fl. Orange		Asp. n.	Asp. n. Bl. Asp.	Asp. n.	Asp. n. Clad. Cit. III	Asp. n. Clad.
P. Gr.	39	Bl. Asp. Syn. Asp. n.	Bl. Asp. Syn.	Syn.	Clad.	O	Asp. n. Orange	Asp. n. Cit. I	Pen. exp. Clad.	Asp. n.
P. Gr.	41	Asp n. Bl. Asp. Syn.	Asp. n.	Asp. n. Clad. Bl. Asp. Asp. fl. II, III Syn.	O	O	Asp. n.	Bl. Asp. Clad. II	Bl. Asp. Syn.	Asp. n.
2nd	42	Asp. n.	Asp. n. Bl Asp.	Asp. n. Clad. II	O	Asp. n. Bl. Asp.	Asp. n. Bl. Asp.	Asp. n. Bl. Asp. Clad. Cit. I	Asp. n. Bl. Asp.	Asp. n.
P. Gr.	44	Bl. Asp. Syn.	Bl. Asp. Syn.	Asp. n. Bl. Asp. Syn. Clad.	Bl. Asp.		Asp. n.	Asp. n. Bl. Asp.	Bl. Asp. Syn.	Asp. n. Bl. Asp.
96°W	45	Bl. Asp.	Bl. Asp II	Asp. n. Bl. Asp. Pen. lut.	O	O	Asp. n. Clad. Bl. Asp. II	Asp. n.	Clad. Orange III	Asp. n. Clad.

Y. C.	50	Asp. n. Clad.	Asp. n. Bl. Asp. III	Asp. n.	Asp. n.		Asp. n. III Asp. fl.	Asp. n. Bl. Asp. II	Asp. n. Clad.	Asp. n. Bl. Asp. Asp. fl. III
P. Gr.	100	Asp. n. Clad.		Asp. n.	O	Asp. n. Clad. Syn.	Syn.	Syn. Asp. n.	Asp. n. Bl. Asp. Syn. Orange	
St. G	101	Asp. n.		Asp. n. Bl. Asp. Orange	O	Asp. n. Clad. Bl. Asp. Orange	Asp. n. Orange	Asp. n. Orange	Clad.	
C. R.	102	Asp. n. Clad.		Asp. n. Bl. Asp.	Clad.	Asp. n. Bl. Asp. Clad.	Asp. n.	Asp. n. Clad.	Asp. n. Bl. Asp. Clad.	
P. Gr.	103	Asp. n. Bl. Asp. Clad. II		Asp. n Clad Bl. Asp. Orange IV		Asp. n. Bl. Asp.	Asp. n. Bl. Asp. Clad. IV Cit. III			Asp. n. Clad. Bl. Asp.
C. R.	104	Asp. n.		Asp. n. Clad. Orange Syn.		Asp. n. Bl. Asp. Syn.	Asp. n. Syn. Cit. III			Asp. n. Bl. Asp.
C. R.	105			Asp. n. Clad.		Asp. n.	Asp. n. Cit. III	Asp. n. Bl. Asp.	Asp. n. Asp fl. Bl. Asp Syn.	
C. R.	106			Asp. n.		O	O	Syn.	O	
C. R.	107			Bl. Asp.		Asp. n. Bl. Asp. Syn. Orange	Asp. n. Bl. Asp. Asp fl. Syn.	Asp. n. Bl. Asp. Orange	Asp. n. Bl. Asp.	
C. R.	108			Asp. n. Clad.		Syn.	O	O	Syn.	
C. R.	109			O		O	O	Syn.	Syn.	

TABLE I—Continued.

ISOLATION OF FUNGI FROM SUGAR.

Type	No.	Amons	Cook	Czapek	Gran. Sugar	Hansen	Kopeloff	Raisin	Smith 10%	Smith 50%
C. R.	110			Asp. n. Clad. Bl. Asp. Orange		Asp. n.	Asp. n. Asp. fl. Orange	Asp. n.	Syn.	
C. R.	111			Orange		Orange IV	O	O	IV	
C. R.	112			Asp. n. Bl. Asp. Clad. Orange		O	Syn.	O	O	
C. R.	113			Asp. n. Bl. Asp.		Asp. n. Bl. Asp.	Asp. n. Bl. Asp.	Bl. Asp. Syn.	Asp. n. Clad. Bl. Asp. Syn. Asp. fl.	
P. Gr.	115	Asp. n. Clad. Bl. Asp. Cit. III III		Clad. Orange IV		Asp. n. Bl. Asp. Orange Clad.	Asp. n. Clad. Bl. Asp. Orange IV, III			Asp. n. Clad. Bl. Asp. Orange
P. Gr.	116	Asp. n. Clad. Bl. Asp.		Bl. Asp. Clad. IV Cit. III		Asp. n. Clad. Bl. Asp. IV, III	Asp. n. Syn. Clad. Bl. Asp.			Bl. Asp. Clad. III
P. Gr.	117	Asp. n. Clad. Bl. Asp. II Syn.		Clad. Bl. Asp. Orange IV Cit. III		Asp. n. Clad. Bl. Asp. Syn.	Asp. n. Clad Orange Syn. III		Asp. n. Clad. Bl. Asp. Orange III	
P. Gr.	118	Clad. Bl. Asp. Orange Cit. III III		Bl. Asp. Clad. Orange Syn. IV		Asp. n. Orange Asp. fl.	Bl. Asp. Clad. Syn. Orange		Clad. Bl. Asp.	

P. Gr.	119	Asp. n. Clad. Bl. Asp.		Asp. n. Clad. Bl. Asp.		Asp. n. Clad. Bl. Asp.	Asp. n. Clad. Bl. Asp.			Asp. n. Bl. Asp.
Y. C.	120	Asp. n. Clad. Bl. Asp. III		Bl. Asp. Clad.		Asp. n. Bl. Asp. Asp. fl.	Asp. n. Clad. Bl. Asp. Orange III Syn.			Asp. n. Clad. Bl. Asp. III
Y. C.	123			Clad. Asp. n. Syn. Asp. f Asp. r. Bl. Asp.			Clad Asp. n. Asp. f. Syn. Bl. Asp. Pen. exp. Asp. r.			
Y. C.	124			Clad. Asp. r. Bl. Asp.			Clad. Bl. Asp. Pen. exp. Asp. n. Syn.			
Y. C.	125			Clad. Bl. Asp. Asp. f. Syn. Asp. n. Pen. exp. Asp. r.			Clad. Bl. Asp Asp. f. Syn. Asp. n. Pen. exp.			
Y. C	126			Clad. Bl. Asp. Pen. exp. Asp. n. III			Clad. Asp. f. Bl. Asp. Pen. exp. Asp. n. Pen ros.			
Y. C.	127			Clad. Asp. f. Bl. Asp. Pen. exp. Syn. Orange			Clad. Asp. f. Bl. Asp. Pen. exp. Orange			

TABLE I—Continued.

ISOLATION OF FUNGI FROM SUGAR.

Type	No.	Amons	Cook	Czapek	Gran. Sugar	Hansen	Kopeloff	Raisin	Smith 10%	Smith 50%
Y. C.	128			Clad. Asp. f. Bl. Asp. Pen. exp. Syn. Asp. n.			Clad. Pen. exp. Bl. Asp. Syn.			
Y. C.	129			Clad. Bl. Asp.			O			
Y. C.	130			Clad. Asp. nid. Asp. r. Asp. n. Asp. f. Pen. exp.			Clad. Asp. f. Bl. Asp. Pen. exp. Asp. n. Cit. III			
Y. C.	131			Clad. Bl. Asp. Asp. n. Pen. div. Asp. sp.			Asp. f. Bl. Asp. Pen. exp. Asp. n. Pen. ros. Asp. sp.			
Y. C.	132			Clad. Asp. f. Bl. Asp. Pen. exp. Asp. nid. Asp n. Monilia n. III			Clad. Asp. f. Bl. Asp. Pen. exp. Monilia n. Asp. nid III			
Y. C.	133			Clad. Bl. Asp. Syn. Asp. n. Monilia n.			Clad. Bl. Asp. Asp. n. Syn.			

Y. C.	134			Clad. Asp. f. Bl. Asp. Asp. nid. Pen. exp. Asp. n. Asp. sp. Syn.			Clad. Asp. f. Pen. exp. Syn. Asp. nid. Asp. n. Pen. div. III			
Y. C.	135			Clad. Bl. Asp. Asp. nid. Asp. f. Asp. n. Asp. sp. Syn. III Pen. exp.			Clad. Asp. f. Pen. exp. Asp. nid. Pen. div. Asp. n.			
Y. C.	136			Bl. Asp. Syn.			Bl. Asp. Syn.			
Stand. G¹ Stand. G² Stand. G³ Stand. G⁴				Asp. n. Bl. Asp. O Bl. Asp. Asp. n.			Clad. Syn. O Syn. Asp. n. Bl. Asp.			

The principal fungi isolated from the sugar samples on hand are indicated in Table I. These tabulated results represent the average of triplicate determinations. The summary of these data is recorded in Table II, which represents a calculation of these results in terms of per cent.

TABLE II.

PER CENT OF SAMPLES SHOWING FUNGUS.

SUGAR TYPE	Total Number of Samples	Aspergillus niger	Cladosporium	Penicillium luteum	Citromyces I	Penicillium divart.	Unknown Sterile II	Unknown Sterile IV	Syncephalastrum	Sterile Orange	Citromyces III	Unknown Sterile III	Blue Aspergillus	Penicillium expansum	Aspergillus flavus
Plantation granulated..........	18	100	100	17	17	6	50	39	50	72	33	61	100	22	22
Plantation granulated undried..	3	100	100	33	0	0	67	33	0	67	0	33	100	33	67
Yellow clarified...............	16	81	94	0	0	19	6	0	63	13	6	38	100	70	75
Standard granulated...........	5	60	40	0	0	0	0	0	40	20	0	0	80	0	0
Cuban raw..................	11	82	64	0	0	0	0	9	73	45	18	0	64	0	36
96°..........................	6	100	83	17	17	0	67	33	33	50	17	50	100	50	67
96° washed..................	10	100	90	10	0	20	70	30	10	20	10	30	100	60	40
2nd.	8	100	100	12	25	0	75	37	12	50	50	50	100	12	60
Av.	REL.	97	90	12	8	7	45	25	38	45	18	35	100	33	44

For example, the second column represents the total number of samples of each sugar type examined. If, as in the case of the Plantation granulated samples, *Aspergillus niger* was isolated in all 18 instances, the per cent of samples showing that fungus to be present would obviously be 100. But where this fungus was found in only 3 out of 5 samples of Standard granulated, the per cent recorded would be only 60. From this Table II it is possible to observe some interesting correlations. While it is difficult to ascribe a definite fungus flora to any particular type of sugar, yet it may be seen that the lower grades of sugar—i. e., ''seconds'' and 96° sugars—show a relatively high degree of infection qualitatively and quantitatively. While the results do not show the Plantation granulated undried to be more infected than dried Plantation granulated sugar, the greater number of samples of the latter presupposes an undue weighting and would lead to the inference that in reality the undried sugar has a relatively greater variety of infection. The fact that the Plantation granulated shows a greater infection than the Yellow clarified series is probably due to the greater age, and consequent exposure to sources of contamination. In accordance with the general expectation, the Standard granulated samples showed the least fungus infection.

The *Blue Aspergillus* and *Aspergillus niger* were isolated from practically every sample of sugar on hand, while the *Cladosporium* was found in 90 per cent of the cases. (The averages were computed on the basis of the *Blue Aspergillus* being rated at 100.) The *Sterile Orange fungus, Unknown Sterile II,* and *Aspergillus flavus* were isolated from about one-half the total number of samples, while the other organisms appeared less frequently in the following order: *Syncephalastrum, Unknown Sterile III, Penicillium exp., Unknown Sterile IV, Citromyces III, Penicillium luteum, Citromyces I, Penicillium divarticatum.* Those fungi which occurred in only rare instances were not tabulated, but are noted in Table III, which represents a list of the species isolated.

Table III.

FUNGI ISOLATED FROM CANE SUGAR.

SERIES I.

Aspergillus niger.
Aspergillus flavus.
Aspergillus nidulans.
Blue Aspergillus.
Citromyces *I, II, III.*
Penicillium expansum (?).
Penicillium divarticatum.
Penicillium luteum ser.
Syncephalastrum.
Trichoderma sp.
Sterile Orange fungus.
Unknown *I, II, III, IV.*

SERIES II.

Aspergillus sp.
Aspergillus, Bur. Chem. No. 3556.
Aspergilus repens.
Penicillium purpurogenum ser. near Penicillium pinophilum.
Penicillium purpurogenum ser near Penicillium luteum, a soil form.
Penicillium luteum—purpurogenum ser. near Penicillium regulosum.
Fusarium sp.
Monilia nigra (Browne).
Monascus purpurens ser.
Dematiaceous fungus.
Unknown *V, VI,* etc.
Penicillium roseum.

Series I comprises the group of fungi which appeared with greater frequency in the samples of sugar examined, while Series II includes the species appearing more infrequently. It will be noted that the species of *Penicillium* and *Aspergillus* are present in greatest number, an observation which is in agreement with that of previous investigators. It may be mentioned, incidentally, that *P. glaucum* is included under *P. expansum* (?) in the recent work of Dr. Thom*[42].

While there was no attempt made to record quantitative results, nevertheless the fact that these organisms appeared on media of abnormally high sucrose content adds evidence to their authenticity as true sugar fungi.

Since the plating method has the obvious limitation of not revealing whether the colony has developed from spores or mycelia, a microscopic examination was made of the samples of sugar under investigation. In many instances mycelia were actually detected, but in the majority of cases the evidence was either negative or doubtful in character. The presence of a considerable amount of foreign organic material, such as strands from the bagging, plant tissues, etc., proved confusing. Mycelia were stained with Conn's[8] Rose Bengal by Dr. Thom and the authors in samples of Cuban raw sugar and inoculated sugars used in our later experimentation, thus proving conclusively the possibility of fungus development, multiplication and activity in such a highly concentrated substratum. (See Plates I and II.)

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* The authors gratefully acknowledge their indebtedness to Dr. Charles Thom and Miss M. Church, of the Bureau of Chemistry, U. S. Department of Agriculture, for these identifications.

TABLE IV.

EFFICIENCY OF DIFFERENT MEDIA IN DEVELOPING VARIED TYPES OF FUNGI.

TYPE	Number	Amons	Cook	Czapek	Gran. Sugar	Hansen	Kopeloff	Raisin	Smith 10%	Smith 50%
P. G.	1	0	60	100	20	0	60	80	20	80
96°	2	100	100	100	50	50	50	50	0	100
P. G.	3	40	20	100	20	40	60	80	20	60
96° W.	7	25	25	100	25	50	50	25	25	25
96° W.	8	50	50	100	0	25	25	75	25	..
96° W.	9	0	25	100	0	50	25	50	25	50
2nd	11	75	25	100	25	75	50	50	25	25
96° W.	12	50	50	100	0	50	25	50	50	75
96° W.	13	40	20	100	0	20	40	20	40	40
96° W.	14	33	33	100	0	67	33	..	100	33
P. G.	15	0	20	60	0	100	80	40	40	40
P. G.	16	20	100	80	20	20	40	40	40	40
P. G.	17	67	67	67	33	0	33	100	67	33
2nd	18	25	25	100	0	50	0	50	25	..
96° W.	19	0	25	100	25	25	50	25	50	25
2nd	20	50	50	100	50	17	85	34	100	85
96°	21	80	40	60	100	20	60	20	60	20
P. G. und.	22	25	75	100	25	0	50	25	0	50
96°	23	50	75	100	25	25	50	50	0	75
2nd	24	40	40	100	40	20	80	40	40	40
P. G. und.	25	17	34	100	0	17	50	50	34	17
96°	26	20	20	100	40	60	60	60	20	20
96° W.	27	0	67	100	33	33	100	67	33	67
P. G. und.	28	20	40	100	20	20	60	20	20	80
P. G.	29	25	50	100	25	25	25	50	0	50
2nd	30	100	67	100	33	67	67	33	33	0
96° W.	31	80	20	100	0	20	0	20	20	40
P. G.	32	20	40	80	20	40	40	40	60	100
96°	33	50	67	100	33	67	83	0	83	33
96° W.	34	75	75	100	25	0	100	25	75	25
P. G.	35	67	33	100	0	0	17	33	17	17

TABLE IV—Continued.

TYPE	Number	Amons	Cook	Czapek	Gran. Sugar	Hansen	Kopeloff	Raisin	Smith 10%	Smith 50%
2nd	36	20	40	100	20	0	40	40	40	60
2nd	37	33	50	100	. .	17	33	17	50	33
P. G.	39	100	67	33	33	0	67	67	67	33
P. G.	41	44	15	100	0	0	15	44	30	15
2nd	42	25	50	75	0	50	50	100	50	25
P. G.	44	50	50	100	25	. .	25	50	50	50
96° W.	45	25	50	75	0	0	100	25	75	50
Y. C.	50	50	75	25	25	. .	75	75	50	100
St. G.	101	25	. .	75	0	75	25	50	100	. . .
C. R.	102	67	. .	67	33	100	33	67	100	. . .
P. G.	103	80	. .	100	. .	40	100	. .	. .	60
C. R.	104	25	. .	100	. .	75	75	. .	. .	50
C. R.	105	. .	. .	50	. .	25	50	50	100	. .
C. R.	106	. .	. .	100	. .	0	0	100	0	. .
C. R.	107	. .	. .	25	. .	100	100	75	50	. .
C. R.	108	. .	. .	100	. .	50	0	0	50	. .
C. R.	109	. .	. .	0	. .	0	0	100	100	. .
C. R.	110	. .	. .	100	. .	25	75	25	25	. .
C. R.	111	. .	. .	50	. .	100	0	0	50	. .
C. R.	112	. .	. .	100	. .	0	25	0	0	. .
C. R.	113	. .	. .	40	. .	40	40	40	100	. .
P. G.	115	83	. .	50	. .	67	100	. .	. .	67
P. G.	116	60	. .	80	. .	100	80	. .	. .	60
P. G.	117	100	. .	100	. .	80	100	. .	100	. .
P. G.	118	100	. .	100	. .	60	80	. .	40	. .
P. G.	119	100	. .	100	. .	100	100	. .	. .	67
Y. C.	120	67	. .	33	. .	50	100	. .	. .	67
Y. C.	123	. .	. .	85	. .	. .	100	. .	. .	. .
Y. C.	124	. .	. .	60	. .	. .	100	. .	. .	. .
Y. C.	125	. .	. .	100	. .	. .	65	. .	. .	. .
Y. C.	126	. .	. .	85	. .	. .	100	. .	. .	. .
Y. C.	127	. .	. . .	80	. .	. .	100	. .	. .	. .
Y. C.	128	. .	. .	100	. .	. .	67	. .	. .	. .
Y. C.	129	. .	. .	100	. .	. .	0	. .	. .	. .
Y. C.	130	. .	. .	100	. .	. .	100	. .	. .	. .
Y. C.	131	. .	. .	85	. .	. .	100	. .	. .	. .
Y. C.	132	. .	. .	100	. .	. .	87	. .	. .	. .
Y. C.	133	. .	. .	100	. .	. .	80	. .	. .	. .
Y. C.	134	. .	. .	100	. .	. .	100	. .	. .	. .
Y. C.	135	. .	. .	100	. .	. .	67	. .	. .	. .
Y. C.	136	. .	. .	100	. .	. .	100	. .	. .	. .
Ave.	REL.	56	56	100	21	46	68	45	54	48

In Table IV is calculated the relative efficiency of the different media employed for yielding a variety of fungi. Thus, if from sample No. 1 (Type P. G.) the greatest number of different fungi isolated on any one medium was 5, and this occurred on Czapek's agar, the latter would receive a rating of 100. Since only 4 different organisms were isolated on Raisin agar, it received a rating of 80, and similarly with the other media on a purely qualitative basis. It will be seen from the general average that the largest variety of fungi was isolated on Czapek's agar, followed in order by Kopeloff (Modified Czapek), Amons, Cook II, Smith 10%, Smith 50%, Hansen, Raisin, and Granulated sugar. However, it is interesting to note that Kopeloff's Modification shows up to far better advantage on the newer samples No. 101-136. For this reason, as well as for the saving of time involved, we have used this agar for all plating in the laboratory when only one medium was being used. The rate of colony development was observed to be most rapid on this agar, a good growth being obtained after 3 days' incubation at 28°-30°C. Amons, Cook II, Czapek, Hansen and Smith 10% developed colonies in 4-7 days' time, while Smith 50% and Granulated sugar required 7-10 days. No definite correlation could be established between the media and organisms for which they were peculiarly adapted. The growth of fungi on Granulated sugar agar was particularly interesting because of the abnormally high sucrose concentration and slight amount of available nutrients.

PART II.

THE DETERIORATION OF CANE SUGAR BY FUNGI IN PURE CULTURE.

Having isolated the fungi which occur in cane sugar and found that they belonged chiefly to the *Aspergilli* and *Penicillia,* and having further established their presence in the mycelial as well as spore stage, it becomes necessary to further define the activities of these organisms. In the brief historical review presented in the introductory portion of this bulletin, mention has been made of the activities of fungi in inverting highly concentrated solutions of sucrose and even sugar itself. Effront[14] has an interesting discussion of the phenomenon based on the work of Duclaux[12], Raulin[35], Wasserzug[45] and others. Therefore, to determine whether or not the fungi isolated and named above were capable of deteriorating sugar, it was essential to carry out the work in pure culture on sterilized sugar. The problem of inoculation was indeed a difficult one because of the strict necessity for performing this operation without the introduction of any moisture. It was planned to add the mycelia and spores of each of the fungi to be studied to each of two widely divergent and representative types of sugar (Plantation granulated and Cuban raw) known to have somewhat above the normal amount of moisture. After incubating for a suitable period of time at an optimum temperature, this sugar was to be used as an inoculum in the succeeding experiments. The first question to arise, obviously, is whether the fungi could be recovered after inoculation. This is answered in the affirmative by the data recorded in Table V, which represent only the average of closely agreeing triplicate determinations.

TABLE V.

MYCOLOGICAL ANALYSIS OF SUGARS INOCULATED WITH FUNGI.

PLANTATION GRANULATED.

Number	Flask	Fungus	Presence		
1	300 cc.	Check	—	—	—
2	300 cc.	Aspergillus niger	+	+	+
6	200 cc.	Check	—	—	—
8	200 cc.	Aspergillus niger	+	+	+
11	200 cc.	Blue Aspergillus	+	+	+
14	200 cc.	Sterile Orange Fungus	+	+	—

TABLE V—Continued.

CUBAN RAW.

Number	Flasks	Fungus	Presence		
16	300 cc.	Check	—	—	—
18	300 cc.	Aspergillus niger	+	+	+
21	300 cc.	Blue Aspergillus	+	+	+
22	200 cc.	Check	—	—	—
24	200 cc.	Aspergillus niger	+	+	+
27	200 cc.	Blue Aspergillus	+	+	+

The method of procedure was as follows: 30 gm. and 50 gm. portions of each sugar were placed in 200 and 300 cc. cotton plugged Erlenmeyer flasks respectively. These sugars were sterilized in the Arnold for half an hour on each of 3 successive days and were shaken thoroughly at the end of each of the two 15-minute sterilization intervals, and finally stirred with a sterile spatula. After 3 days' incubation at 26°C. the contents of each flask were examined for sterility by plating on Kopeloff's agar.

Pure cultures of *Aspergillus niger, Blue Aspergillus,* and *Sterile Orange* fungus grown on Raisin agar were thoroughly shaken with 3-5 cc. of Raulin's solution, and 3 large platinum loopfuls of each added to the desired flask. The flasks were shaken thoroughly and incubated at room temperature for one week. At the end of this period they were plated on Kopeloff's agar. Thus in Table V it will be seen that there was a perfect recovery in every inoculated flask except one containing the *Sterile Orange* fungus, which was especially difficult to handle. The check flasks remained sterile.

With this as a starting point, a number of 250 gm. portions of Plantation granulated and Cuban raw sugars were placed in 1000 cc. Erlenmeyer flasks and sterilized in the Arnold for half an hour on 3 successive days. After 3 days' incubation at 28°C. they were sterilized again for 1 hour in the Arnold and incubated at room temperature until inoculated. This time no moisture was added, but mycelia and spores were scraped with a sterile platinum scoop from the surface of a large number of Petri dishes, in which the agar was covered with an abundant pure growth of the desired organism. The flasks were incubated at room temperature and shaken thoroughly every day for 3 weeks. Counts were then made on Kopeloff's agar and the results recorded in Table VI.

TABLE VI.

NUMBER OF ORGANISMS IN INOCULATED SUGARS.
PLANTATION GRANULATED.

Number	FUNGUS	1-1000 Dilution Colonies per Plate		Av. Number per 1 gm.	1-8000 Dilution		Av. Number per 1 gm.
1	Aspergillus niger...	50	47	49,000	..	..	......
2	Sterile Orange......	0	0	1,000	..	..	......
3	Cladosporium	10	16	13,000	..	..	......
4	Penicillium No. 2694	250	250	250,000	30	33	256,000
5	Blue Aspergillus....	175	192	184,000	20	21	168,000
6	Aspergillus flavus...	15	13	14,000	2	1	12,000
7	Syncephalastrum ...	0	0	1,000	..	..	......
8	Check	0	0	....	..	..	......
	CUBAN RAW.						
10	Aspergillus niger...	170	150	160,000	..	..	......
11	Sterile Orange.....	0	0	1,000	..	..	......
12	Cladosporium	0	0	1,000	..	..	......
13	Penicillium No. 2694	22	20	21,000	..	..	......
14	Blue Aspergillus...	325	500	413,000	78	84	648,000
15	Aspergillus flavus...	90	85	88,000	..	..	......
16	Syncephalastrum ...	0	0	1,000	..	..	......
17	Check.	0	0	....	..	..	......

It may be mentioned, parenthetically, that it is always our custom to incubate dilution flasks with the plates for comparison as to growth, as the results prove of interest.*

It will be seen that the average number of organisms per gm. of inoculum varied from 1,000 to 648,000, and usually 10 gm. of each inoculum were added to 150 gm. of sterile sugar, which proved to be satisfactory.

In conducting an experiment involving the inoculation of sterile sugar with pure cultures of fungi, it was of interest to note that the prevailing conception among mycologists with regard to the limit of concentration for the growth of *Penicillia* and *Aspergilli* has generally been considered to be between 60-70 per cent. The work of Eschenhagen[15], Raciborski[34] and others may be cited in this connection. As Thiele[41] has pointed out, the temperature boundaries of these organisms depend upon the nutrients present, as well as the varying concentration and reaction of the medium. It was established by this investigator that with increasing concentrations the temperature limits are increased. Thus where the optimum temperature for *Penicillium* is generally about 25°C. where a 50 per cent sugar solution was employed the organism grew well at 34°C. A similar 10 per cent increase is obtained with *Aspergillus niger.* Duclaux[12] and Wasserzug[45] have shown that *Aspergillus niger, Penicillium glaucum* and many other fungi are capable of inverting sucrose. The former claims that 35°C. is the optimum temperature for *Aspergillus niger,* which corroborates Raulin's[35] limits of 19-43°C. for this organism. Moreover, the latter regards humidity as directly affecting the growth of the fungus, as well as the influence of various nutrients. In the light of such considerations it was planned to incubate all inoculated flasks at 30°C. The sugars employed in this experiment designed to study the deteriorative activity of pure cultures of fungi isolated from sugar were of the three principal types, namely: Plantation granulated, Refined and Cuban raw. One hundred and fifty gm. portions were weighed into sterilized 500 cc. Erlenmeyer flasks and sterilized as usual and examined for sterility. To each of three flasks of each type of sugar 10 gm. of inoculum containing *Aspergillus flavus, Aspergillus niger, Blue Aspergillus, Syncephalastrum,* and sterile sugar alone, respectively, were added. An inoculum of Cuban raw sugar was used for the Cuban raw series, while Plantation granulated was used for both the Plantation granulated and the Refined series. The inoculated flasks were incubated at 30°C. for 4 months, with no provision for replacing any of the moisture which evaporated. Under such circumstances the sugars dried out to such an extent as to make the moisture content negligible. At the end of the incubation period, the contents of each flask were analyzed and the results recorded in Tables VII-IX.

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* In this connection, it is a pleasure to thank Mr. W. L. Owen, who has volunteered many valuable suggestions.

Before discussing these data, however, it is well to bear in mind the general concensus of opinion, both in the laboratory and the field, to the effect that the "Factor of Safety" is a fairly reliable criterion for the keeping quality of a sugar. In other words, it is generally conceded that

$$\frac{\text{Moisture}}{100 - \text{Polarization}}$$

should yield a fraction lower than .33 to guarantee the non-deterioration of an unwashed raw sugar. Browne[7] more recently has shown that for Cuban and Porto Rican sugars this constant is a little too high and the value 0.3 is more nearly correct. However, for washed 96-test sugars this factor becomes of less value. Furthermore, according to Owen[32], it is inapplicable to white sugars "because in such cases it is the total moisture that must be considered, since the density of the film can not exceed the maximum concentration in which microorganisms can destroy sucrose."

TABLE VII.

ANALYSES OF SUGAR INOCULATED WITH FUNGI.

PLANTATION GRANULATED.

Number	FUNGUS	*Presence after 4 months	S. Pol.	Clerget	Reducing Sugars	Moisture	Moisture ratio
7	Check	—	99.2	99.26	0.16	0	
10	Check	—	99.0	98.88	.16	0	
14	Check	—	98.9	98.73	.15	.07	
AVE.		—	99.0	98.96	.16	.02	0.02
12	Aspergillus flavus. . . .	—	99.1	99.06	.16	.02	
13	Aspergillus flavus. . . .	—	98.9	98.88	.16	0	
AVE.		—	99.0	98.97	.16	.01	0.01
16	Blue Aspergillus.	+	98.9	98.27	.17	.11	
17	Blue Aspergillus.	+	98.6	98.50	.16	.02	
18	Blue Aspergillus.	—	99.2	99.12	.15	.07	
20	Blue Aspergillus.	—	99.1	99.22	.16	.12	
AVE.		±	98.9	98.78	.16	.08	0.07
15	Syncephalastrum	—	99.0	98.88	.13	.08	
21	Syncephalastrum	+	98.9	98.66	.14	.05	
23	Syncephalastrum	—	98.9	98.94	.15	.08	
25	Syncephalastrum	—	98.9	98.59	.15	.05	
AVE.		∓	98.9	98.77	.14	.07	0.06
4	Aspergillus niger. . . .	—	99.0	99.06	.18	0	
8	Aspergillus niger. . . .	—	99.4	99.25	.15	0	
28	Aspergillus niger. . . .	+	98.9	98.59	.13	.10	
29	Aspergillus niger. . . .	+	98.8	98.43	.15	.04	
AVE.		±	99.0	98.84	.15	.03	0.03

* Average of triplicate plates with two dilutions.

The column marked "Moisture" in Table VII indicates the presence of a very low moisture content and the "factor of safety" or "moisture ratio" in this series would be considerably below 0.10. Therefore, marked deterioration would not be anticipated. That such is actually the case may be seen in the single polarization (S. Pol.) sucrose Clerget (Clerget) and reducing sugars values set forth. Practically all these determinations are within the limit of experimental error. Slight indications of the loss of sucrose are perhaps to be noted in the case of the *Blue Aspergillus* (flasks 16 and 17) and *Aspergillus niger* (flasks 28 and 29). The same general negative evidence is to be gathered from Table VIII.

TABLE VIII.

ANALYSES OF SUGAR INOCULATED WITH FUNGI.

REFINED.

Number	FUNGUS	Presence after 4 months	S. Pol.	Clerget	Reducing Sugars	Moisture	Moisture ratio
2	Check	—	99.4	99.72	.09	.04	
3	Check	—	99.7	99.84	.05	.02	
5	Check	—	99.4	99.38	.05	.02	
6	Check	—	99.4	99.49	.04	.03	
AVE.			99.5	99.61	.06	.03	0.06
7	Aspergillus flavus. . . .	+	99.3	99.33	.05	.03	
8	Aspergillus flavus. . . .	+	99.5	99.76	.05	0	
AVE.		+	99.4	99.54	.05	.02	0.03
14	Blue Aspergillus.	+	99.5	99.90	.04	.03	
15	Blue Aspergillus.	+	99.5	99.46	.04	.05	
16	Blue Aspergillus.	+	99.3	99.26	.04	.04	
17	Blue Aspergillus.	+	99.3	99.16	.04	.04	
AVE.		+	99.4	99.44	.04	.04	0.07
18	Syncephalastrum	+	99.6	99.61	.04	0	
19	Syncephalastrum	—	99.7	99.80	.04	0	
21	Syncephalastrum	+	99.2	99.04	.04	0	
23	Syncephalastrum	+	99.3	99.30	.06	.0	
AVE.		+	99.4	99.44	.05	0	0
26	Aspergillus niger. . . .	+	99.7	99.72	.04	0	
27	Aspergillus niger. . . .	+	99.7	99.80	.04	0	
28	Aspergillus niger. . . .	—	99.7	99.91	.05	0	
29	Aspergillus niger. . . .	+	99.6	99.65	.05	0	
AVE.		+	99.7	99.77	.05	0	0

Only the slightest signs of inversion are exhibited by *Blue Aspergillus* (16 and 17) and *Syncephalastrum* (21 and 23).

TABLE IX.

ANALYSES OF SUGAR INOCULATED WITH FUNGI.

CUBAN RAW.

Number	FUNGUS	Presence after 4 months	S. Pol.	Clerget	Reducing Sugars	Moisture	Moisture ratio
1	Check	—	95.7	96.27	1.04	0.83	
2	Check	—	95.7	95.99	1.09	1.06	
4	Check	—	95.6	95.97	1.04	1.15	
AVE.		—	95.7	96.08	1.06	1.01	0.26
17	Aspergillus flavus. . .	—	95.6	95.93	1.09	1.07	
18	Aspergillus flavus. . .	—	95.6	95.78	1.09	1.06	
AVE.		—	95.6	95.86	1.09	1.07	0.24
6	Blue Aspergillus. . . .	+	95.0	95.51	1.13	0.96	
7	Blue Aspergillus. . . .	—	95.0	95.51	1.04	1.00	
8	Blue Aspergillus. . . .	+	95.1	95.54	1.56	1.14	
10	Blue Aspergillus. . . .	—	95.2	95.74	1.19	0.94	
AVE.		+	95.1	95.58	1.23	1.01	0.20
12	Syncephalastrum . . .	+	95.7	96.27	1.09	1.11	
13	Syncephalastrum . . .	+	94.8	95.28	1.09	1.06	
15	Syncephalastrum . . .	+	95.4	95.86	1.09	1.10	
16	Syncephalastrum . . .	+	95.6	96.02	1.09	0.99	
AVE.		+	95.4	95.86	1.09	1.06	0.23
26	Aspergillus niger. . . .	+	95.0	95.51	1.11	1.05	
27	Aspergillus niger. . . .	—	95.6	96.01	1.11	1.23	
28	Aspergillus niger. . . .	—	95.5	95.93	1.11	1.22	
29	Aspergillus niger. . . .	+	95.6	96.01	1.14	1.29	
AVE.		—	95.4	95.86	1.12	1.20	0.26

However, in the Cuban raw series shown in Table IX, where the moisture content is appreciable, though well below the factor of safety limit, some evidence of the activity in inverting sucrose may be found in the case of the *Blue Aspergillus* and to a lesser degree in *Aspergillus niger.* This experiment consequently may be regarded as establishing the lower limit of the deterioration of manufactured cane sugar by the fungi employed. While a factor of safety of 0.1-.20 is practically a guarantee of non-deterioration in the above sugars, nevertheless, even at this abnormally high concentration there appears to be some slight evidence of possible activity on the part of these organisms. The bearing which this has upon the general factor of safety rule will be dealt with in more detail in a subsequent experiment in Part II.

All the sugars were plated in triplicate on Kopeloff's agar, using 1 cc. of dilution (20 gm. sugar to 40 cc. 17° Brix sugar solution) to 8 cc., and the presence or absence of the inoculated fungi indicated by plus and minus signs, respectively, in the third column of the foregoing tables. There is little consistency with regard to the recovery of these fungi and there is, furthermore, no indication of any multiplication of the organisms involved. A microscopical examination failed to establish the presence of mycelia in any of these sugars.

TABLE X.

ANALYSES OF SUGAR INOCULATED WITH DIFFERENT FUNGI.

PLANTATION GRANULATED.

Number	FUNGUS	Presence after 4 months	Single Pol.	Clerget	Reducing Sugars	Moisture	Moisture ratio	*Dry Basis*	
								S. P.	S. C.
103	Check	—	99.6	99.58	0.14	0.09	.22	99.7	99.7
20	Cladosporium	+	98.7	98.86	0.25	0.19	.15	98.8	99.0
21	Aspergillus flavus. .	+	99.0	98.73	.15	.06	.06	99.0	98.8
22	Blue Aspergillus. . .	+	98.5	98.54	.44	.19	.13	98.6	98.7
23	Penicillium 2694. . .	+	98.9	98.82	.20	.22	.20	99.1	99.0
24	Syncephalastrum. . .	+	99.3	99.23	.14	.06	.09	99.4	99.3
24A	Unknown I.	—	99.3	99.20	.17	.08	.10	99.1	99.3
25	Trichoderma	+	98.7	98.65	.31	.20	.15	98.9	98.8
26	Citromyces	+	98.7	98.75	.14	.03	.02	98.7	98.8
27	Asp. nidulans.	+	98.8	98.87	.15	.09	.07	98.9	98.9
28	Citromyces II.	+	99.2	99.07	.14	.07	.10	99.3	99.1
29	Pen. pinophilum. . .	—	98.8	98.83	.28	.23	.20	99.0	99.0
30	Citromyces III. . . .	—	99.0	99.22	.15	.11	.17	99.1	99.3
31	Penicillium div. . . .	+	99.5	99.54	.19	.09	.06	99.6	99.6
32	Unknown II.	+	98.4	98.29	.14	.11	.06	98.5	98.4
33	Sterile Orange.	—	99.1	98.76	.16	.07	.07	99.2	98.8
34	Unknown III.	+	98.9	98.92	.23	.15	.13	98.9	99.1
36	Aspergillus niger. . .	+	99.5	99.50	.15	.06	.04	99.6	99.6
37	Unknown IV.	+	97.4	97.39	.31	.31	.12	97.7	97.7
38	Unknown V.	—	98.9	98.77	.31	.30	.27	99.2	99.1

In Table X are presented the analyses of the sugars inoculated with the various fungi isolated and which were to be used whenever required for inoculation. In this Plantation granulated series the moisture ratio was uniformly low and the amount of deterioration, in consequence, was relatively slight, as will be seen from the values of sucrose Clerget on the dry basis (which represent the most accurate criteria for comparison). Some of the *Unknown Sterile fungi,* notably II, and the *Blue Aspergillus* caused an appreciable inversion. It will be noted, furthermore, that the *Blue Aspergillus* is responsible for the greatest amount of reducing sugars throughout the series.

Table XI.

ANALYSES OF SUGAR INOCULATED WITH DIFFERENT FUNGI.

CUBAN RAW.

Number	Fungus	Presence after 4 months	Single Pol.	Clerget	Reducing Sugars	Moisture	Moisture ratio	*Dry Basis*	
								S. P.	S. C.
104	Check	—	96.2	96.24	1.00	1.00	.26	97.2	97.2
20	Cladosporium	+	95.9	95.74	1.09	1.07	.26	97.0	96.8
21	Aspergillus flavus..	+	96.0	95.93	1.09	1.26	.31	97.2	97.1
22	Blue Aspergillus...	+	92.3	91.07	2.28	2.28	.30	94.5	93.2
23	Penicillium 2694...	+	96.2	96.11	0.98	1.04	.27	97.2	97.1
24	Syncephalastrum ..	+	95.9	95.81	1.09	1.18	.29	97.0	96.9
24A	Unknown I........	—	96.2	96.11	1.09	1.33	.35	97.5	97.4
25	Trichoderma	+	96.2	96.11	1.09	1.35	.35	97.5	97.4
26	Citromyces I......	+	95.5	95.44	1.11	1.55	.34	97.0	96.9
27	Asp. nidulans......	+	96.2	96.13	1.09	1.26	.33	97.4	97.3
28	Citromyces II......	—	96.1	96.05	1.14	1.23	.32	97.3	97.2
29	Pen. pinophilum...	—	96.2	96.13	1.09	1.26	.33	97.4	97.3
30	Citromyces III.....	—	96.0	95.89	1.09	1.16	.29	97.1	97.0
31	Penicillium div....	+	95.9	95.75	1.09	1.00	.24	96.9	96.7
32	Unknown II.......	+	96.0	95.75	1.09	1.30	.32	97.2	97.0
33	Sterile Orange.....	—	95.9	95.75	*	1.43	.35	97.3	97.1
34	Unknown III......	+	94.9	95.06	1.11	1.44	.28	96.3	96.4
36	Aspergillus Niger...	+	96.1	96.05	1.09	1.33	.34	97.4	97.3
37	Unknown IV......	+	96.0	96.01	1.11	1.29	.32	97.3	97.3
38	Unknown V.......	+	95.6	95.74	1.56	1.68	.38	97.2	97.4

* Lost.

In the same way, in the Cuban raw series shown in Table XI, where the moisture ratio was considerably higher than in the preceding series, a considerable inversion as indicated by the low Clerget value was obtained with the *Blue Aspergillus.* Correspondingly, the greatest value for reducing sugars was noted for the same organism. In a similar manner a number of other fungi were more active in the Cuban raw than in the Plantation granulated series, accentuating again the importance of moisture as a limiting factor in the deterioration of sugar by micro-organisms. The results obtained with these pure cultures substantiate the more general conclusions arrived at by Browne[7] and Owen[32] that deterioration in raw sugar takes place when the moisture factor is between 0.25 and 0.30. In fact, there are ample grounds for regarding the activity of fungi as proceeding at higher concentrations than any of the other groups of micro-organisms previously studied, and later experiments will show that the factor of safety rule is not reliable where there is a considerable infection due to the presence of fungi. The mycological observations carried out by the plating method previously described revealed the presence of fungi in a larger number of instances than in the preceding experiment (Tables VII-IX), where the moisture content was decidedly lower.

Having established the lower limit for the deterioration of sugar by the fungi isolated and witnessed the stimulation in activity of these organisms where the moisture content was increased, it became desirable to approximate more nearly the optimum conditions for the full exercise of their power in this direction. The following experiment was conducted in a manner similar to that previously described, with one paramount difference, namely, the sterilized sugars (of the three principal types) were permitted to absorb moisture in the autoclave before inoculation. This was accomplished in the following manner. As previously, 150 gm. portions of Plantation granulated, Refined and Cuban raw sugars were placed in 500 cc. sterilized Erlenmeyer flasks and sterilized in the usual manner, with frequent shaking to prevent caking. It was observed that if the sugar was shaken by tapping the flask against the hand from the time it was removed from the Arnold until it was thoroughly cool, there would rarely be any lumps formed. Only flasks free from fungus contamination and having the slightest bacterial content were used. The flasks were then placed in the autoclave, and the cotton plugs removed. Several small pans of boiling water were placed inside, as well as wads of cotton soaked in boiling water. The autoclave was heated sufficiently to permit the sugars to become slightly warmed and they remained in the "humidor" for 4 hours. From time to time the water in the pans was brought to the boiling point and the autoclave heated slightly. The sugars were analyzed for moisture and the process continued until the desired moisture ratio was obtained with each sugar. The initial moisture ratio of these sugars averaged about 0.45.

The method previously described for preparing an inoculum was improved upon in the following manner. Pure cultures of each fungus to be employed were grown on numerous plates of Kopeloff's agar several weeks before use, to obtain an abundant spore production. Large samples of Plantation granulated and Cuban raw sugar were sterilized in the usual way. Then in the sterile chamber (used only for careful bacteriological work) successive portions of sugar from a single flask would be introduced into the Petri dishes containing the desired organism, shaken slightly and returned to the flask through a sterile aluminum funnel. In this way the whole mass of sugar in the flask received a liberal coating of spores and thorough shaking resulted in a uniform distribution. These flasks containing the inoculum were incubated together with uninoculated sugar (to be added to the check flasks) at 30°C. for 2 weeks and shaken daily. Ten gm. portions of inoculum were used in inoculating the sterile sugars previously described. Before inoculation, however, a count of spores in each inoculum was made by making a 1 to 2 dilution and examining with a Zeiss blood counting cell as used by us[23] in another connection. These data are to be found in Tables XV-XVII.

All flasks containing sterilized sugars made up to optimum moisture content were inoculated in triplicate with 10 gm. portions of the inoculum containing the desired organism (uninoculated portions of the same sugar being used for the check flasks) and incubated at 30°C. for one month. The humidity of the incubator was maintained at 65-75 per cent. At the end of the incubation period, which was relatively short, the contents of all flasks were analyzed and the averages of closely agreeing triplicate determinations recorded in Tables XII-XIV.

Table XII.

ANALYSES OF SUGAR INOCULATED WITH FUNGI.

PLANTATION GRANULATED.

Fungus	Mycelia	S. P.	S. Clerget	Reducing Sugars	Moisture	Moisture ratio	*Dry Basis*	
							S. P.	S. C.
Check	—	98.6	98.3	0.23	.44	.31	98.9	98.8
Aspergillus flavus.........	—	98.5	98.3	0.26	.47	.27	98.9	98.7
Blue Aspergillus..........	+	97.1	97.2	0.69	.88	.31	97.9	98.0
Syncephalastrum	—	98.6	98.5	0.24	.37	.26	98.9	98.9
Aspergillus niger.........	∓	97.4	97.4	0.44	.65	.27	98.1	97.9

It will be readily observed in Table XII that the *Blue Aspergillus* and *Aspergillus niger* are responsible for considerable inversion in Plantation granulated sugar, as represented by the reduction in Clerget values on the dry basis when compared with the check. There is a deterioration of almost 1% in this short period. That this is a true inversion is corroborated by the tripling and doubling, respectively, in the amount of reducing sugars. The moisture ratio in this series approaches the critical value arrived at in the valuable contributions of Browne[7], Owen[32] and others. There is, consequently, no doubt that both the *Blue Aspergillus* and *Aspergillus niger* at moisture ratios of 0.31 and 0.27 respectively are capable of inducing a serious loss in Plantation granulated sugar. Two of the other organisms, *Aspergillus flavus* and *Syncephalastrum,* on the other hand, exhibit little if any tendency to cause inversion, even under these circumstances. It is especially noteworthy in this connection to emphasize the fact that the two fungi capable of producing marked deterioration are the same which appeared most frequently in the large number of various sugar samples examined and discussed in Part I of this bulletin, namely, the *Blue Aspergillus* and *Aspergillus niger.*

TABLE XIII.

ANALYSES OF SUGAR INOCULATED WITH FUNGI.

REFINED.

FUNGUS	Mycelia	S. P.	S. Clerget	Reducing Sugars	Moisture	Moisture ratio	*Dry Basis*	
							S. P.	S. C.
Check	—	99.6	99.4	.11	.16	.35	99.7	99.6
Aspergillus flavus	+	98.2	98.2	.24	.42	.23	98.6	98.6
Blue Aspergillus	+	97.9	97.9	.57	.63	.30	98.6	98.3
Syncephalastrum	—	99.4	99.4	.22	.19	.34	99.6	99.6
Aspergillus niger	—	98.8	98.8	.41	.51	.41	99.3	99.3

The Refined series recorded in Table XIII yields much the same evidence. Here, again, where the moisture ratio is in the vicinity of 0.3 and deterioration is to be expected, the *Blue Aspergillus* and the *Aspergillus niger* are active in inversion, as evidenced by the low Clerget and high reducing sugar values. *Aspergillus flavus* also appears to have some activity in the present series. This fungus caused a loss of 1 per cent of sucrose, while the *Blue Aspergillus* was responsible for a still greater inversion. *Aspergillus flavus* produced this striking deterioration at a moisture ratio of only 0.23, while the *Blue Aspergillus* operated at 0.30.

TABLE XIV.

ANALYSES OF SUGAR INOCULATED WITH FUNGI.

CUBAN RAW.

FUNGUS	Mycelia	S. P.	S. Clerget	Reducing Sugars	Moisture	Moisture ratio	*Dry Basis*	
							S. P.	S. C.
Check	—	95.1	95.2	0.97	1.98	.40	97.0	97.2
Aspergillus flavus	—	95.2	95.2	1.05	1.90	.40	97.0	97.1
Blue Aspergillus	—	92.7	93.4	2.01	2.50	.34	95.0	95.6
Syncephalastrum	—	95.0	94.9	1.01	2.03	.40	96.5	96.8
Aspergillus niger	—	94.6	94.8	1.09	2.01	.37	96.5	96.7

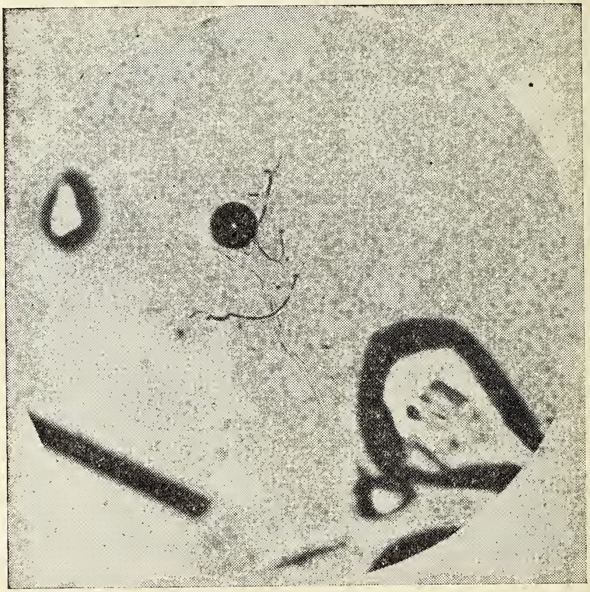

PLATE I.

Mycelial threads of *Blue Aspergillus* in center of the field adjoining crystals of Plantation granulated sugar, from inoculated flask, after one month's incubation.

Photomicrograph enlarged four times from original taken with 16 mm. objective.

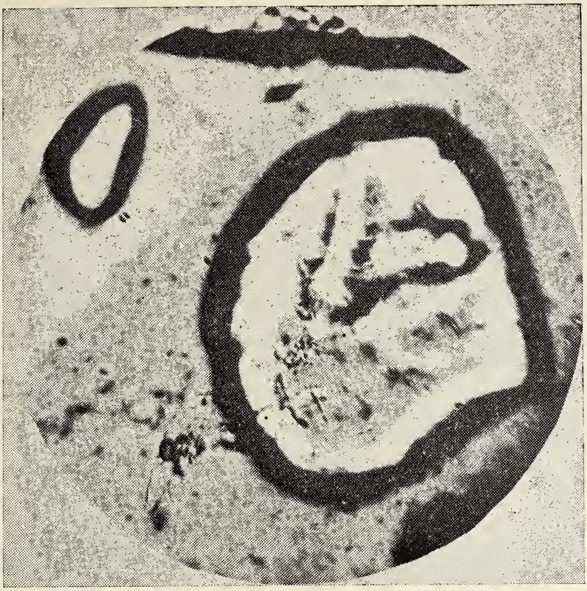

Plate II.

Mycelial threads on and near large crystals of Cuban raw sugar. Uninoculated sample, collected on arrival from Cuba.

Photomicrograph enlarged four times from original taken with 16 mm. objective.

In the Cuban raw series in Table XIV the results parallel those previously noted. Again the *Blue Aspergillus* and *Aspergillus niger* are very active, the former causing a loss of 1.6 per cent sucrose Clerget on the dry basis and proving this to be a true case of inversion by a doubling in the amount of reducing sugars. *Syncephalastrum* exhibits some inclination to invert the sucrose present. In this series the moisture ratio was such as to warrant the sugar "unsafe" and it is patent that the fungi in question are capable of taking advantage of optimum environmental conditions.

In comparing the activity of the *Blue Aspergillus* throughout the three types of sugar it will be seen that the maximum loss of sucrose occurred in the Cuban raw sugar, and that this coincides with the greatest moisture ratio, 0.34. In fact, the individual instance of greatest loss of sucrose throughout the experiment is to be found in flask 9 of this series, which shows a loss of 2.4 per cent in the Clerget on the dry basis and a correspondingly high value for reducing sugars. The Refined series showed a loss of 1.3 per cent Clerget on the dry basis, with a moisture ratio of 0.30. It is altogether probable that the deterioration was greater in this than in the Plantation granulated series (which had practically the same moisture ratio and caused a loss of only 0.8 per cent) because of the lower density of the hygroscopic film about each sugar particle. This involves a discussion of the validity of the factor of safety for washed sugars.

It has been previously pointed out that the reliability of this factor decreases with the artificial dilution of the hygroscopic film induced by washing the sugar. The polarization of sugar is increased by washing, which means a proportional increase in purity, hence a removal of solids non-sucrose from the film. This implies a film of lower density and consequently results in a greater tendency towards deterioration. Browne[7] states the matter succinctly in a discussion of deductions from the "factor of safety" rule. He claims that "if a fixed ratio between moisture and non-sucrose is the governing factor in the keeping quality of raw cane sugars, there are a number of deductions or corollaries which must follow from such a proposition.

The first corollary which we will consider is that slight fluctuations in moisture content have a much greater influence upon the keeping quality of high grade than of low grade sugars. Thus 0.1 per cent increase in moisture will raise the factor of a 99° sugar* with 0.28 per cent moisture from 0.28 to 0.35, but will raise the factor of a 90° sugar of 2.80 per cent moisture from 0.28 to only 0.29. In other words, a high-grade sugar of good keeping quality can be made unfit for storage by the absorption of only 0.1 per cent moisture, while the keeping quality of a low-grade sugar having the same factor will not be sensibly affected. This conclusion is abundantly confirmed not only by laboratory tests but by practical experience. The storage of high-grade raw sugars or of moist refined sugars has always been regarded as hazardous. *Even white granulated sugar has been found to deteriorate in a humid atmosphere, owing to the absorption of moisture.*†

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* Typographical error corrected in correspondence.

†The italics are ours.

This is precisely the condition we achieved in this experiment through the activity of the fungi in pure culture.

The significant feature of the factor of safety rule, from the biological standpoint, is that it attempts to define the limit of concentration beyond which microorganisms cannot operate, and consequently sucrose is not destroyed by inversion. The data which were presented in Tables VII-XIV, however, when considered as a whole, make it evident that the factor of safety for white sugars is very close to 0.1, while for Cuban raw sugar it is nearer 0.20, where appreciable fungus infection is the limiting factor. While it is undoubtedly true that fungi will not grow in dry sugar, nevertheless the moisture required to stimulate their activity is remarkably slight in amount. The nature and extent of the fungus infection are indubitably predisposing factors in any sensible deterioration of sugar. In the light of these researches, then, it would be deemed advisable to revise our conception of the factor of safety rule in such fashion as to make adequate provision for fungus infection, which appears more destructive than any other single group of microorganisms in sugar yet studied. Experiments are now in progress to define more rigorously the limits of concentration for the activity of these fungi and the results will be available in the near future.

Before commencing the chemical analyses indicated in Tables XII-XIV a mycological examination was made by plating out a portion of the contents of each flask. Ten gm. portions were dissolved in 40 cc. of 17° Brix sugar solution, 1 cc. being plated on Kopeloff's agar, and incubated at 30°C. for 5 days. It will be seen from Table XV that all the inoculated flasks of the

TABLE XV.

MYCOLOGICAL EXAMINATION OF INOCULATED SUGARS.

PLANTATION GRANULATED.

After One Month Incubation.

FUNGUS	Dup.	Dup.	Average	Average Per Gm.	Average Per Gm. Original Inoculation
Check	2X	2X	2X	8X	0
Aspergillus flavus	118	136	127	638	1000
Blue Aspergillus	766	800	873	3915	10,000
Syncephalastrum	N	N	N	N (3X)	40
Aspergillus niger	1033	933	983	4916	8,000

X—Contaminating organism.
N—Covers plate.

Plantation granulated series indicated the presence of an abundant number of colonies of the fungus used. The average ranges from 638 to 4916 per gm. The check flasks show a negligible contamination. The original inoculation recorded in the last column represents the number of spores found by using the Zeiss blood-counting cell[23]. Obviously the direct microscopic count is always higher than the plate count. This has been clearly demonstrated in the work of Conn[8]. Therefore the apparent discrepancy between the original and final count is quite negligible. One outstanding fact, however, is that there is no indication of multiplication on any large scale. The same general results were obtained with the Refined series as shown in Table XVI. The number of colonies found is in close agreement with the count made in the previous series, the only marked difference occurring with *Aspergillus niger*. Again there is no evidence of any considerable multiplication.

Table XVI.

MYCOLOGICAL EXAMINATION OF INOCULATED SUGARS.

REFINED.

After One Month Incubation.

FUNGUS	Dup.	Dup.	Average	Average Per Gm.	Average Per Gm. Original Inoculation
Check	0	0	0	0	0
Aspergillus flavus	102	100	101	505	1000
Blue Aspergillus	800	966	883	4115	10,000
Syncephalastrum	N	N	N	N	40
Aspergillus niger	316	316	316	1580	8,000

X—Contaminating organism.
N—Covers plate.

Table XVII.

MYCOLOGICAL EXAMINATION OF INOCULATED SUGARS.

CUBAN RAW.

After One Month Incubation.

FUNGUS	Dup.	Dup.	Average	Average Per Gm.	Average Per Gm. Original Inoculation
Check	2X	3X	3X	15X	0
Aspergillus flavus	11	9	10	52	13,300
Blue Aspergillus	0	0	0	0	64,000
Syncephalastrum	N	X	X	5X	8,000
Aspergillus niger	246	280	263	1315	38,400

X—Contaminating organisms.
N—Covers plate.

The results obtained in the Cuban raw series are incorporated in Table XVII, and in general the count is much lower than in the previous sets. The most striking feature is the great reduction in numbers as compared with the original inoculation. If anywhere, it is in this series that the greatest number of organisms would be expected, because of the higher moisture content. Especially baffling is the fact that no colonies of *Blue Aspergillus* were to be found and the sugar in the flasks containing this fungus showed the greatest deterioration. Possibly some error in the plating was responsible, but, unfortunately, the flasks were exposed to contaminating influences before that operation could be repeated. As before, the direct microscopic count is significantly larger than the final plate count.

A microscopical examination was made of the contents of each flask, taking several 1 gm. portions. The authors were assisted in this by Dr. Charles Thom's visit to this laboratory. He not only made numerous microscopical examinations of inoculated and uninoculated samples of sugar, but was generous enough to go over all cultures of fungi mentioned in Table III, in addition to spending some time on the factory problems at hand. The presence of mycelia was established (by staining with Conn's[8] Rose Bengal) in several instances where deterioration had occurred (see Plates I and II). This is indicated by the plus and minus signs in the third column of Tables XII-XIV. Thus, in Table XII, deterioration occurred in flasks 7, 8 and 9 where mycelia were present, etc. In no case was the presence of mycelia unaccompanied by evidence of deterioration. However, in other instances where deterioration was noted no mycelia could be detected, and only spores had been present. Such is the case in Table XIV, flasks 7, 8 and 9, where the greatest amount of deterioration in any one series is recorded.

This phenomenon is worthy of further study (which is at present being carried forward*), for upon its interpretation depends much that is of economic significance. Since it is possible for the spores of fungi to secrete enough invertase to cause the deterioration of sugar without the development of mycelia, then sugar which has been properly dried and considered "safe" by virtue of its moisture ratio, would in reality be likely to undergo deterioration, depending upon the nature and extent of the infection. The fact that fungus spores alone may cause deterioration points indubitably, from a new angle, to the necessity for cleanliness in the sugar mill. It leads to an investigation now in progress of sterilizing sugar in the centrifugals or reducing the infection by adequate protection at this point. From the broader viewpoint, it is of prime importance to industrial mycology to have determined to what extent the spores of fungi are capable of secreting enzymes without active growth, and it is hoped that the experiments now being conducted in this laboratory will throw some light on this question. For the present it may serve to interpret some interesting phenomena. For example, in a recent conversation Dr. C. A. Browne has suggested that it appeared to be a plausible explanation of the fact which he has often noted, that some sugars which start to deteriorate at a high moisture ratio, continue to do so in spite of the lowering of this ratio below the critical point of safety. Similarly, Dr. C. L. Alsberg regarded such an interpretation as bearing on the yield of penicillic acid under anærobic conditions by an ærobic fungus, *Penicillium puberulum*[1]. Instances could be multiplied indicating the application of this phenomenon to other scientific and industrial fields. The bearing which it might have upon the factor of safety rule is of more immediate concern. It has been previously mentioned in connection with the earlier inoculation experiments that the moisture ratio for inhibiting fungus activity was found to be considerably lower than for other microorganisms. No mycelia were found in the sugars analyzed in Tables VII-IX; therefore, it is reasonable to suppose that the spores were responsible for whatever slight deterioration was observed. It becomes more imperative than ever, in the light of these data, to consider a revision of the factor of safety rule from the biological standpoint of fungus infection.

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* While awaiting publication, we have succeeded in establishing experimentally that the spores of *Aspergillus niger* contain invertase, by inoculating 10 and 20 per cent sugar solutions with spores alone and obtaining a 5 per cent reduction in polarization with 10 cc. of heated spore suspension, and 10 per cent reduction with double this number of spores (i. e., 20 cc.) in 24 hours. There was a proportional increase in reducing sugars.

PART III.

FUNGI IN THE SUGAR FACTORY.

Having isolated the fungi which occur in marketable cane sugars and determined their power to cause deterioration by inversion, it was of practical significance to trace the fate of the fungi and bacteria through the process of sugar manufacture. With this in view, a daily bacteriological and mycological examination of each stage in the process was carried on throughout the past grinding season. In brief, the local process consists of crushing the harvested cane to extract the raw juice. This is treated with sulfur dioxide fumes and later limed back to slight acidity and brought to a boil. The clear juice resulting from this clarification is, after settling, run into a storage tank. The solid impurities are put through a filterpress—the filtered juice joining the clarified juice in the storage tank. The juice is evaporated to syrup in the "effects" by heating at about 120-185°F., under a vacuum of 10-26 inches of mercury. The syrup is taken to the "vacuum pan," where the sugar is grained at 130-165°F., under a vacuum of 22-25 inches of mercury. The massecuite, as usual, is run into the centrifugal and washed. Further methods of refinement, other than the use of vegetable carbon experimentally, were not employed in our sugar mill.

The samples were collected in sterilized, cotton-plugged, 300 cc. Erlenmeyer flasks and the effort was made to complete the plating within 1 hour of their arrival to the laboratory, the samples being kept in the refrigerator until used. A sterilized 18° Brix sugar solution was used as dilution water throughout. One cc. of each of the following dilutions was plated in duplicate on Kopeloff's agar (Czapek was used for comparison in the last runs): No dilution, Dilution A, 20 cc. in 180 cc. of dilution water constituting 1-10 dilution; Dilution B, 10 cc. of A in 90 cc. constituting 1-100 dilution; Dilution C, 10 cc. of B in 90 cc. constituting 1-1000. Sterilized 10 cc. and 1 cc. pipettes were used in the usual manner.

In Tables XVIII-XXIII are presented the averages of the duplicate counts for each dilution, each table representing what is known as a "run," and includes 5 "clarifiers." Obviously, the clarifiers lose their identity in the vacuum pan and only one set of figures is obtainable for the succeeding stages in each run. In order not to further complicate the data presented, the average of Dilutions A, B and C is represented by a single column "Dil." It must be stated that the agreement between these dilutions was not all that could be desired, but it was natural to expect wide discrepancies where such a concentrated material was under observation. The data as presented give an adequate cross-section of what may be found in a small Louisiana mill. No attempt was made to classify the bacteria found, but they may be said to approximate qualitatively and quantitatively the species described by Owen[29]. (In fact, the heading "Bacteria" includes all microorganisms other than fungi.)

TABLE XVIII.

MICROORGANISMS IN THE FACTORY.

Run 1.

Clarifier	FUNGI	JUICES					Syrup	Masse cuite	SUGAR				Cent. Air	
		Raw	Sulph	Limed	Filt	Settled			Raw	Washed	Molasses	Wash Water	Above	Below
2	No. Dil..........	NS NAf	7S	0	22BA	0	38BA 60C1 5II 3Ci	NC1 12S	NC1 BA Af	160C1 50BA 3II	10BA	0	14C1 7BA	17C1
	Dil.............	1000S 2300 Af 50T	10S 40Af	0	30BA	0	280BA 115C1 400Ci	400BA 310C1		200C1 50BA 30P				
	Bact...........	*1000	*70	5	*28	*10	2200	340	?	400	500	175	33	10
3	No. Dil.........	8BA 7T 3III	0	0	0	BA Ci	BA							
	Dil.............	4300T 1300BA 1000II	0	0	0	10 BA	0							
	Bact...........	*400	*30	65	N	N	19							

4	No. Dil.	S,T	0	0	S	0	0								
	Dil.	55S 250BA	0	0	0	0									
	Bact.	*800	1200	0	N	N	225								
5	No. Dil.	29BA 9S	S	0	3S	2S									
	Dil.	2500BA 10S	0	0	0	0									
	Bact.	*400	190	220	350	380									
Av. No. Dil.		9BA 27S 2T	2S	0	5BA,S	0									
Av. Dil.		269S 1087BA 1087T 250 II 575Af	3S 10Af	0	7BA	2BA									
Av. Bact.		*650	*25	73	*14	5190	814								

Table XIX.

MICROORGANISMS IN THE FACTORY.

Run 2.

Clarifier	FUNGI	JUICES					Syrup	Masse cuite	SUGAR		Molasses	Wash Water	Cent. Air	
		Raw	Sulph	Limed	Filt	Settled			Raw	Washed			Above	Below
1	No. Dil.	0	0		0		0	0	31C1 2BA S	230C1 BA S	3BA 7C1	0	BA	C1 BA S Af. Ci.
	Dil.	0	0		0		0	0	100C1 10BA 10S	0	80C1 10BA	0		
	Bact.	*800	*7		225		200	180	85	800	110	2500	4	14
2	No. Dil.	0	0		0		0							
	Dil.	0	0		0		0							
	Bact.	*1500	?		40									
3	No. Dil.	0	S		0									
	Dil.	25S												
	Bact.	*270	?		*12									
4	No. Dil.	0	0											
	Dil.	0	0											
	Bact.	*1000	3300		770									
5	No. Dil.	0	2S											
	Dil.	105S	425BA											
	Bact.	N	N											
Av. No. Dil.		0	S	0										
Av. Dil.		26S	85BA											
Av. Bact.		*792	5150											

TABLE XX.

MICROORGANISMS IN THE FACTORY.

Run 3.

Clarifier	FUNGI	JUICES					Syrup	Masse cuite	SUGAR				Cent. Air	
		Raw	Sulph	Limed	Filt	Settled			Raw	Washed	Molasses	Wash Water	Above	Below
1	No. Dil.	0	0	0	0	0	Or. 2C1	S 4BA 5C1	S 37C1	S 2BA 12C1	15C1 20 2BA	Or. 0	5C1	S 10C1
	Dil.	0	0	0	0	0	0	40BA 30C1	10S 70C1 30BA	10S 60C1	110C1 30BA	0	0	0
	Bact.	N	N	30	19	270	80	275	65	120	270	1600	14	180
2	No. Dil.	0	BA	0										
	Dil.	0	15BA	0										
	Bact.	*1000	2250	220										
3	No. Dil.	25BA 22Ci	2BA	0	Or.	C1 P	S BA							
	Dil.	850BA	0	0	0	0	10BA 10S							
	Bact.	*1000	5000	N	290	10	100							

TABLE XX—Continued.

MICROORGANISMS IN THE FACTORY.

Clarifier	FUNGI	JUICES					Syrup	Masse cuite	SUGAR		Molasses	Wash Water	Cent. Air	
		Raw	Sulph	Limed	Filt	Settled			Raw	Washed			Above	Below
4	No. Dil.	18BA S 3Ci	0	S Af.	S 3P	0								
	Dil.	1200BA 10S	0		0	0	0							
	Bact.	*220	430		90	130	5000							
5	No. Dil.	8BA	4S	10S	0	BA	BA C1							
	Dil.	3700BA 4000T	10S	0	0	10BA	0							
	Bact.	*425	4000	*10	5500	60	130							
Av. No. Dil.		10BA 5Ci	S BA	2S	0	P	C1 BA							
Av. Dil.		1150BA 2S 800T	2S 4BA	0	0	2BA	2BA 2S							
Av. Bact.		*661	3143	3417	1475	118	1327							

TABLE XXI.

MICROORGANISMS IN THE FACTORY.

Run 4.

Clarifier	FUNGI	JUICES					Syrup	Masse cuite	SUGAR				Cent. Air	
		Raw	Sulph	Limed	Filt	Settled			Raw	Washed	Molasses	Wash Water	Above	Below
1	No. Dil.........	2S 11BA	5S	0	S	S	0	S	4An. NS	4An. 3S	0	0	3Af.	4C1 Or. Syn.
	Dil.............	100S 100BA *3Af.	10BA 20S	0	10S	0	0	10S	35S	10An. 20BA 10S	0	0	0	0
	Bact...........	*400	8500	80	400	550	180	1000	460	310	1730	750	2	8
2	No. Dil.........	NAf.	S	0	0	0	0							
	Dil.............	310Af.	0	0	0	0	0							
	Bact...........	*1000	8000	50	20	55	55							
3	No. Dil.........	8S NAf. 6BA	0	0	0	0	0							
	Dil.............	*3Af. 500S	0	0	0	0	0							
	Bact...........	*800	0	5	*10	1120	60							

TABLE XXI—Continued.

MICROORGANISMS IN THE FACTORY.

Clarifier	FUNGI	JUICES					Syrup	Masse cuite	SUGAR		Molasses	Wash Water	Cent. Air	
		Raw	Sulph	Limed	Filt	Settled			Raw	Washed			Above	Below
4	No. Dil.........	NAf. 4S	0	0	0	0	0							
	Dil.............	2500Af. 20S 100Ci	0	0	0	0	0							
	Bact...........	*750	1200	35	*7	12	100							
5	No. Dil.........	NAf. 5S	0	0	0	5Pd.	0							
	Dil.............	*2Af. 1100S *2Or.	0	0	0	0	0							
	Bact...........	*1000	*4	40	68	0	6							
Av. No. Dil.................		4S 3BA NAf.	2S	0	S	Pd.	0							
Av. Dil.		344S 25BA 2200Af. 25Ci 400Or.	4S 2BA	0	2S	0	0							
Av. Bact....................		*790	4300	42	3500	346	80							

TABLE XXII.

MICROORGANISMS IN THE FACTORY.

Run 5.

Clarifier	FUNGI	JUICES					Syrup	Masse cuite	SUGAR				Cent. Air	
		Raw	Sulph	Limed	Filt	Settled			Raw	Washed	Molasses	Wash Water	Above	Below
1	No. Dil.	6Af. 2BA 5Pd. S					0	0	7S	2S	S	0	An. P Ci	Af. 2Ci 1S 3An. 2P 20C1
	Dil.	1500Af. 70S 120BA 100An.					0	0	0	0	0	0	0	0
	Bact.	*123					430	2200	1400	1450	*9	200	3	7
2	No. Dil.	8S 15BA NAf.	0		0	0								
	Dil.	1000S 200BA *8Af.	0		0	0								
	Bact.	*300	1300		0	0	0							
3	No. Dil.	NAf. An. BA S, T					0	0						
	Dil.	150S 2200Af. 800BA 200Ar.					80							
	Bact.	*1000												

TABLE XXII—Continued.

MICROORGANISMS IN THE FACTORY.

Clarifier	FUNGI	JUICES					Syrup	Masse cuite	SUGAR		Molasses	Wash Water	Cent. Air	
		Raw	Sulph	Limed	Filt	Settled			Raw	Washed			Above	Below
4	No. Dil.	0	0		0		0							
	Dil.	0	0		0		0							
	Bact.	*1000	1900		700		160							
5	No. Dil.	5S NAf.	9S		0		0							
	Dil.	2000S 2000Af.	60Af. 10S		0		0							
	Bact.	*1500	5000		1500		600							
Av. No. Dil.		NAf. 4BA 3S	3S		0		0							
Av. Dil.		2640Af 645S 229BA 25An. 50Ar.	20Af. 3S		0		0							
Av. Bact.		*800	2730		733		254							

Table XXIII.

MICROORGANISMS IN THE FACTORY.

Run 6.

Clarifier	FUNGI	JUICES					Syrup	⅔ SYRUP AND ⅓ MOLASSES				Wash Water	Cent. Air	
								Masse cuite	SUGAR					
		Raw	Sulph	Limed	Filt	Settled			Raw	Washed	Molasses		Above	Below
1	No. Dil.........	NAf. 20S	0	0	0	0	0	S	BA	3An. 8Cl BA	P S			
	Dil.............	*5Af. *2BA	0	0	0	0	0							
	Bact...........	*600	2100	10	190		120	600	73	30	175			
2	No. Dil.........	NAf. 2P 10Ar.	2S	0	0		2An.							
	Dil.............	2500Af. 500S 1100 III	10S				10An.							
	Bact...........	*600	4500	200	900		1250							
3	No. Dil.........	NAf. NS												
	Dil.............	*2Af. *3S												
	Bact...........	*800	*10	6	35		130							

TABLE XXIII—Continued.

MICROORGANISMS IN THE FACTORY.

Clarifier	FUNGI	JUICES					Syrup	Masse cuite	SUGAR				Cent. Air	
		Raw	Sulph	Limed	Filt	Settled			Raw	Washed	Molasses	Wash Water	Above	Below
4	No. Dil.	NAf. NS	NS	0	0		0							
	Dil.	*3Af. *8S	0	0	0		0							
	Bact.	*1000	*7	5	620		750							
Av. No. Dil.		NAf. NS 2Ar.	S	0	0		0							
Av. Dil.		3100Af. 500BA 220,III	2S				2An.							
Av. Bact.		*750	5900	55	438		562							

KEY: *—Thousands omitted.
N—Too numerous to count.
Af—Aspergillus flavus.
An—Aspergillus niger.
Ar—Aspergillus repens.
BA—Blue Aspergillus.
Cl—Cladosporium.
Ci—Citromyces.
Or—Sterile Orange Fungus.
P—Penicillium.
Pd—Penicillium divarticatum.
S—Syncephalastrum.
T—Trichoderma.
I, II, III—Unknown Sterile I, II, III.

In discussing the results presented in Tables XVIII-XXIII, it will be seen that Run 1 had perhaps the greatest amount of fungus contamination. This would be expected because of the fact that the mill had not been in operation for about one year prior to this time. The *Blue Aspergillus* appears with greatest frequency, both qualitatively and quantitatively, in this run and maintains the same tendency throughout the remainder of the season. This is especially significant since it links up with the chain of evidence already gathered which indicates that this fungus is not only found more frequently than any other in sugars of various types but has, moreover, a greater deteriorative power. While the fungus flora of each factory depends necessarily upon a variety of factors, nevertheless we are convinced that even though the *Blue Aspergillus* may not be the predominant organism in every factory, it is present universally. For example, we have found it in two of the largest and cleanest factories in Louisiana. Waksman[44] corroborates the work of Werkenthin[46], which indicates that the *Aspergilli* are predominant in southern soils. Noel Deerr in a recent correspondence suggests that it is reasonable to suppose that fungus infection from the soil may accompany the cane to the mill, and that such infection may be further disseminated by the boots of field workers, etc. This is in accordance with our observations, and represents a factor worthy of serious consideration. It may be added in this connection that Dr. Thom has found an *Aspergillus* to be the main agent in the destruction of certain highly concentrated foodstuffs. *Cladosporium*, likewise, is prevalent under the local conditions, and it is to be found not only through the data in the tables under consideration, but also in abundance on the woodwork, walls, floors, etc., of the mill. We have found the drip-board of the filterpress literally covered with this organism and the *Sterile Orange* fungus. *Syncephalastrum* has appeared somewhat more frequently than was to be expected.

Run 2, shown in Table XIX, had 1 per cent of rice hull carbon added (based on the weight of the juice). It is difficult to account for the absence of fungi in the raw juice of this run, as well as the first 2 clarifiers of Run 3, as shown in Table XX, and no explanation has suggested itself beyond the possibility of some unconscious error in technique.

In Run 5, which received 0.7 per cent rice hull carbon, it will be observed that no fungi appeared in the filtered and settled juices. It is altogether reasonable to suppose that the finely divided carbon mechanically removed some of the microorganisms. Beginning with clarifier 3 of this series, Czapek's agar was used to the end of the season, the results comparing favorably with those previously obtained on Kopeloff's modification, which required a shorter incubation period.

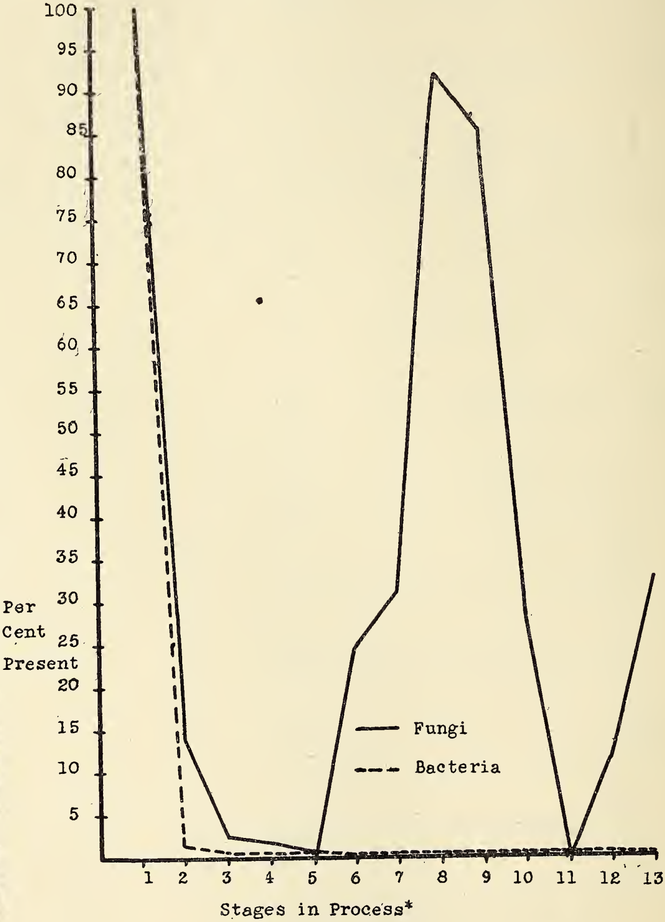
In Run 6, shown in Table XXIII, the syrup of clarifiers 1-5 was augmented in the vacuum pan by the addition of molasses (1/3) taken from previous runs. The results are similar to those recorded in other runs.

Table XXIV.

SUMMARY OF MICROORGANISMS IN THE FACTORY.

RUN		JUICE					Syrup	Masse-cuite	Raw Sugar	Washed Sugar	Molasses	Wash Water	CENT. AIR	
		Raw	Sulph.	Limed	Filt.	Settled							Above	Below
1	Fungi	15	1	0	5	0	53	61	51	100	5	0	11	9
	Bact.	100	3.9	.01	2.2	0.8	0.4	.05	?	.06	.07	.003	.0005	.0001
2	Fungi	0	0		0		0	0	5	100	5	0	.001	.02
	Bact.	100	.71		.034		.028	.026	.011	.112	.013	.24	.0005	.0015
3	Fungi	50	40	5	0		35	32	100	40	50	0	15	15
	Bact.	100	.45	.50	.18	.20	.001	.042	.01	.02	.042	.26	.002	.027
4	Fungi	100	4	2	0	0	0	2	100	15	0	0	6	16
	Bact.	100	.61	.006	.47	.05	.012	.13	.062	.043	.23	.10	.002	.001
5	Fungi	100	4		0		0	0	14	4	2	0	6	58
	Bact.	100	.35		.09	0.3	.05	.29	.17	.17	1.1	.02	.000	.008
6	Fungi	100	1	0	0	0	1							
	Bact.	100	0.8	.007	.08	.07								
Ave.	Fungi	100	13.6	3.3	1.6	0	24.6	31.1	91.8	85.2	28.0	0	11.5	32.8
	Bact.	100	1.14	0.17	0.51	0.28	0.10	0.11	.06	.08	.27	.13	.001	.006

FIG. I



*Stages in Process.

1. Raw Juice.
2. Sulfured Juice.
3. Limed Juice.
4. Filtered Juice.
5. Settled Juice.
6. Syrup.
7. Massecuite.
8. Raw Sugar.
9. Washed Sugar.
10. Molasses.
11. Wash Water.
12. Air above Centrifugal.
13. Air below Centrifugal.

The data in Tables XVIII-XXIII are summarized in Table XXIV, where relative percentages are recorded. Thus the maximum number of organisms appearing in any stage is taken as 100, and the number appearing in the other stages calculated on this basis. A graph of these results is shown in Fig. 1. It is at once evident that the greatest number of microorganisms occurs in the raw or normal juice. The sulfitation causes a decrease of 86 per cent of fungi, while the completion of the clarification process renders the juice practically sterile. However, when the syrup and massecuite are exposed to the air, and especially when the latter enters the centrifugal, reinfection takes place. The fungus content of the syrup appears to be higher than might be expected, but it will be noted that the infection from Run 1, already discussed, served to unduly weight the average. The rapidly whirling centrifugal sucks air from below at great speed and any microorganisms on the floor or in the mill at large have an opportunity of gaining an entrance. This is corroborated by the fact that the air below the centrifugal has approximately 3 times the number of fungi to be found in the air above the centrifugal. The bacterial counts (including microorganisms other than fungi) follow the same general trend as the fungi. There is a greater reduction in numbers, through sulfitation, there being a reduction of 99 per cent, while the clarification process renders the juice practically sterile. The subsequent reinfection does not seem to be as serious as in the case of the fungi. While these results confirm similar ones reported by Owen[29], nevertheless the fact that we were not employing a strictly bacterial medium may account for slight differences.

It is to be regretted that it was impossible at this time to conduct similar exhaustive surveys of the fungus and bacterial flora in each stage of the manufacturing process in other mills, for the limitations attending the observations under the local conditions are clearly recognized.

PART IV.

PRACTICAL DEDUCTIONS.

At the outset of this bulletin the economic significance of the problem at hand was indicated. The fact that the deterioration of sugar in transportation, handling and storage is a result of biological agencies has never been questioned. Therefore, the paucity of extensive research made it advisable to undertake a study of the presence and activity of the fungi, a group of microorganisms which appeared likely to prove of considerable importance. A survey of a large variety of sugars indicated the presence of fungi in significant numbers. The Standard granulated sugars were comparatively free from fungi and infection seemed to be correlated with an increasing moisture content, the lower grades of sugar carrying the largest number and variety of fungi. As has been pointed out by other investigators, the keeping quality of a sugar depends upon moisture as one of its controlling factors. It is of prime importance, then, to reduce the moisture content as much as possible and avoid the subsequent access of moisture.

Since it has been shown that reinfection takes place on a large scale after the massecuite leaves the vacuum pan, it would be advisable to cover the mixer which conveys the massecuite to the centrifugals. This might very easily be accomplished by means of an adjustable and removable canvas or tarpaulin. In this way all dirt, dust and foreign matter, as well as microorganisms, could be excluded, and one of the sources of infection removed. Since the principal infection occurs at the centrifugal and before the sugar is bagged, it is of practical importance to take special precautions at this point. Cleanliness in the factory has been shown to be not only desirable but profitable. The returns are not questionable, but may be measured in dollars and cents. So long as the deterioration of sugar was ascribed to the activity of bacteria alone it was difficult to explain the beneficial effects of cleanliness in the factory, owing to the extreme resistance to heat, etc., of the spores. Owen[32] raises the question: "How, then, could cleanliness eliminate the causative agencies in sugar deterioration?" This query was answered by the discovery of the presence of fungi in sugar which have greater deteriorative powers than bacteria, but which have a low thermal death point and powers of resistance[43], and consequently can be successfully eliminated. And it is in and about the centrifugals that cleanliness is of paramount importance. If possible, the centrifugals should be in a room instead of in the open factory. Whether this is done or not it is essential that there be a concrete floor below the centrifugals. This should be kept clean throughout the day and a swabbing with a hot 0.5 per cent solution of formaldehyde would do much towards destroying contaminating influences. In the off season it would be decidedly advisable to sterilize the mill thoroughly. Where water is used in washing sugar, strict regard must be paid to its purity, for, as is often the case, where the water is contaminated with microorganisms, such a practice is equivalent to an inoculation of sugar with deteriorative organisms. There is no question but that closed centrifugals would be advantageous.

The possibility of actually sterilizing the sugar and washing by means of superheated steam, etc., in the centrifugals is now being studied. That the handling of sugar after it leaves the centrifugal requires care is self-evident, and the condition of the bags must not be disregarded in any consideration of infection. The proper storage of sugar requires a cool, dry, well-ventilated warehouse, preferably with a concrete floor, the sugar being piled on a false floor of boards to permit a thorough circulation of air. It is necessary to guard against all sources of contamination, thereby reducing the infection, which means the elimination of one of the principal factors in deterioration. With the increased interest in the results of scientific experimentation manifested by practical men, it is hoped that some progress will be made towards improving the keeping quality of sugar by insisting upon cleanliness in the factory and maintaining suitable conditions for storage. While the data presented in this bulletin are somewhat technical in character, nevertheless the results have been interpreted as above, from the practical standpoint, and the primary interest has always been in the application of these biological principles to the sugar industry.

In conclusion, the authors are appreciative of the assistance they have so generously received from: Dr. C. A. Browne, whose suggestions and advice have been invaluable in the inter-

pretation of much of the data; Mr. W. L. Owen, with whom frequent consultations brought material aid; Dr. Charles Thom, whose visit cleared up many difficulties; Assistant Director W. G. Taggart and Dr. F. W. Zerban, for their constant help; Mr. E. C. Freeland, for assistance in chemical analyses; Mr. J. McFetridge, of the American Sugar Refinery; Mr. J. C. Murphy and other members of the New Orleans Sugar Exchange; the Angola Plantation and other managements have very kindly supplied us with sugar for experimental purposes. The authors are grateful for the kindness of Dr. Foster M. Johns, of the Tulane University Medical School, in preparing the accompanying photographs, which required considerable time and painstaking effort.

SUMMARY.

1. The fungi isolated on a variety of media from a wide range of cane sugars belonged chiefly to the *Aspergilli* and *Penicillia.* *Aspergillus niger* and a *Blue Aspergillus* occurred in practically all samples.
2. A microscopic examination revealed fungus mycelium in some sugars.
3. Czapek's agar had the greatest relative efficiency of all the media studied for isolating a variety of fungi. The authors' modification showed to good advantage and was responsible for a more rapid colony development.
4. Sterilized sugars inoculated with pure cultures of fungi deteriorated rapidly where the moisture content was appreciable. Little, if any, deterioration occurred when the moisture content was reduced to a minimum.
5. The factor of safety for sugars well infected with fungi would appear to be lower than is generally supposed. Slight evidences of deterioration occurred in Plantation granulated sugar with a factor of 0.1 and in Cuban raw sugar with a factor of 0.20.
6. Fungi spores, as such, contain invertase. These same fungi were responsible for the inversion of sucrose where only spores were present, as well as where mycelia were developd.
7. The fungus which appeared with greatest frequency in all sugars, the *Blue Aspergillus,* also had the greatest deteriorative power.

8. Fungi and bacteria were traced through each stage of the manufacturing process and found in greatest numbers in the raw juice. The clarification process was in effect a sterilization. Reinfection took place in the massecuite and in and about the centrifugals.
9. The practical deductions involve cleanliness throughout the factory, especially in the vicinity of the centrifugals, and adequate storage facilities.

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Baton Rouge

Studies on the Preparation of Vegetable Decolorizing Carbons for the Cane Sugar Industry

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PREFACE.

This laboratory has for the past two or three years been engaged in a study of decolorizing carbons, their preparation, properties and uses. These three phases of the subject are necessarily interdependent, the properties of the carbons being naturally affected by different methods of preparation, and the uses depending largely on the properties. Much attention has, of course, been paid by us to the use of carbons in the cane sugar industry, because this part of the problem is of most immediate concern to the sugar manufacturer. Two previous station publications, Louisiana Bulletins Nos. 161 and 165, deal particularly with this subject. But the preparation of decolorizing carbons is also of much importance to the industry, and a large part of our time between grinding seasons has been devoted to its study. The present bulletin gives the practical results of our work on this question, and our further investigations will be largely based on them.

Our thanks are due to Professor W. R. Dodson, Director of the Experiment Stations, for the great interest he has taken in the progress of our work in all of its phases. It is a pleasure also to acknowledge gratefully the valuable advice so generously supplied by Mr. W. G. Taggart, Assistant Director of this station, and other members of the staff, as well as by Mr. W. L. Owen, of the Penick & Ford Co. We further wish to express our thanks to Dr. Wilder D. Bancroft, Lieutenant Colonel, C. W. S., U. S. A., and to Dr. Geo. A. Hulett, of Princeton University, who very kindly read the manuscript and offered a number of valuable criticisms and suggestions.

I. METHODS OF PREPARATION OF DECOLORIZING CARBONS FROM ORGANIC MATERIALS BY IMPREGNATION WITH MINERAL SUBSTANCES.

Several years ago, when a new vegetable decolorizing carbon, sold under the trade name of Norit, first attracted the attention of the sugar planters of Louisiana, this station began a study of methods for the preparation of carbons derived from plant sources. Our principal object in undertaking this work was to ascertain the effect of differences in the methods used on the decolorizing power of the resulting carbon, and at the same time to secure a carbon of the highest possible decolorizing power for a study of the properties of these carbons. It stands to reason that such a carbon would exhibit other physical, and perhaps chemical, properties, responsible for or related to its decolorizing power, to a higher degree than carbons of low efficiency, and that it would therefore best serve the purpose of an investigation on the underlying causes of decolorizing power. The practical side of the problem has also not been lost sight of, and we have been constantly on the lookout for materials which were abundant and promising as cheap sources of good carbons.

Ordinary wood charcoal usually exhibits only a very slight decolorizing power, but certain methods for increasing this effect were devised by some French chemists almost one hundred years ago.[1] These methods consisted in heating the charcoal or raw material itself in the presence of other "impregnating" substances. Since that time only sporadic attempts seem to have been made to follow up this question, and it was not until the appearance on the market, about the year 1911, of Eponit and Norit, both of them made by processes held secret, that chemists again began to pay greater attention to vegetable decolorizing carbons. Most of the work done along this line has been published in the form of patent specifications. The following Table I contains a short resumé of the most important literature on the subject, based on a compilation by Schneller,[2] to which we have added some further references, mostly of recent date. Those cases in which animal matters are used as raw materials have been left out. The list is incomplete, because in many cases we could not consult the original articles, but it gives a general idea of the methods employed by the authors. The different steps of the processes are arranged in tabular form, in order to facilitate comparison.

TABLE I.

Authors and Year	Reference	Raw Material	Impregnating Substance	Temperature Final Heating	After-Treatment
1. F. Garcin 1878	Sugar Cane 1878, 104	Wood, rags, paper, etc.	H_2SO_4		
2. A. Dubosc, 1897	Chem. Absts. 1908, 3280	Sulfite Pulp	Raw Material impregnated		
3. L. de Percival 1897	Chem. Absts. 1908, 3280	Dye wood residues	HCl		HCl
4. R. V. Ostrejko 1900	Z. Zuckerind, 1902, 66	Wood, etc.	CaO, calcium acetate, etc.	Red heat	HCl
5. C. Halse and A. Klingelhofer, 1903	Z. Zuckerind, 1903, 1104	Wood, straw, sawdust, peat, lignite, etc.	H_2SO_4		
6. Karlik and Stanek, 1903	Oest.-Ung. Z. 1903, 256	Filter press cake from beets	$CaCO_3$		
7. R. Goldstein 1909	Chem. Absts. 1911, 1835	Earthy lignite	Clay	Red heat	
8. A. Lotz, 1911	Chem. Absts. 1912, 2983	Coal, lignite	Alkalis, alk. carbonates, etc.		
9. A. Lotz, 1911.	Chem. Absts. 1913, 246	Lignite	None	Up to 1200°C.	
10. F. Richter 1911.	Chem. Absts. 1911, 3921	Spent decolg. carbon	Iron compounds, etc.		
11. J. V. Kruszewsky, 1912.	Chem. Absts. 1914, 997	Peat	Alkalis, alk. carbonates, CaO, BaO		
12. Matheus, 1912.	Chem. Absts. 1912, 1365	Waste Sulfite liquor	Raw Material impregnated		HCl
13. O. Molenda and J. Wunsch, 1912	Z. Zuckerind. 1912, 1379	Sawdust, etc.	Chlorine Gas		
14. L. Pilaski, 1912.	Chem. Absts. 1914, 818	House and street refuse	CaO	600-700°C.	HCl
15. Richter and Richter, 1912.	Z. Zuckerind. 1912, 1378	Cellulose material	None, dry dist. at 200-300°C.	600-700°C.	
16. Richter and Richter, 1912.	Chem. Absts. 1912, 1518	Coal dust	Clay, chalk, lime, ashes, iron oxide		
17. Richter and Richter, 1912.	Chem. Absts. 1912, 1518	Carbonaceous material	Brown coal ashes		
18. Richter and Richter, 1912	Chem. Absts. 1912, 2149	Carbonaceous material	Mineral plus nitrogenous org. matter, etc.		
19. F. W. Spanutius, 1912.	Int. Sugar J. 1915, 338	Argol waste	Raw material impregnated		
20. C. G. Hanhart, 1913.	Chem. Absts. 1915, 133	Vegetable material	$CaCl_2MgCl_2$	350°C.	H_2O

TABLE I — Continued.

Authors and Year	Reference	Raw Material	Impregnating Substance	Temperature Final Heating	After-Treatment
21. G. H. Hellseng, 1913.	Int. Sugar J. 1915, 195	Culm (earthy lignite)	Dry distillation		
22. Fritz Pott, 1913.	Int. Sugar J. 1915, 194	Albuminoid substances	Mineral matter		
23. A. Scholtz, H. Koch, B. Henatsch, 1913.	Z. Zuckerind. 1914, 68	Peat, wood, bone	Superheated steam	Red heat	
24. Alphons Zelniczek, 1913.	Chem. Absts. 1915, 130	Wood, sawdust, etc.	$ZnCl_2$	Distilling pt. of $ZnCl_2$	HCl
25. W. Clacher 1914.	Chem. Absts. 1914, 2635	Cane bagasse	Raw material impregnated		NaOH HCl
26. Ralph H. McKee, 1914.	Int. Sugar J. 1915, 338	Wood	NaOH, Black ash		HCl
27. J. Wunsch 1914.	Chem. Absts. 1915, 2801	Wood, sawdust, etc.	$ZnCl_2$		H_2O HCl
28. W. A. Beatty, 1915.	Off. Gaz. U. S. Pat. Off. 1915	Gums, etc.	Porous substances		
29. F. Friedrich, 1915.	Chem. Absts. 1916, 539	Beet molasses			
30. A. H. Bonnard, J. E. Rule, Jas. Nichol, 1917.	Int. Sugar J. 1917, 241, 339	Saw dust	CaO, $CaCO_3$	1000°C. or over	HCl
31. R. H. Catlin, 1917.	Chem. Absts. 1917, 1733	Residue from shale distn.	Raw material impregnated		HF
32. R. A. Demme, 1917.	Chem. Absts. 1918, 410	Hydrolyzed wood waste, peat, etc.	Alk. earth carbonates		HCl
33. Ensuiko Seito Takushoku-Kabushiki Kaisha, Y. Okada and H. Miyake, 1917.	Chem. Absts. 1918, 2670	Water grasses or pith from same, Cane bagasse	CaO	750-800°C.	HCl
34. A. Knoepflmacher, 1917.	Chem. Absts. 1918, 1112	Waste sulfite liquor	Alkalis, alk. earths and their carbts.	Red heat	H_2O HCl SO_2
35. R. V. Ostrejko, 1917.	Chem. Absts. 1917, 2534	Carbonaceous material	Superh. steam Uncomb. gases	White heat	
36. U. Tokunaga, 1917.	Chem. Absts. 1918, 520	Rice hulls, Veg. wax resds. potato refuse	$MgCl_2$		HCl
37. T. Hayashi and U. Emura, 1918.	Chem. Absts. 1918, 1843	Spent decolg. carbon	Raw material impregnated		H_3PO_4 SO_2
38. H. L. Lyon and S. S. Peck, 1918.	Int. Sugar J. 1918. 431	Cane molasses with kieselguhr, etc.	H_2SO_4	Gentle heat	H_2O
39. R. W. Mumford, 1918.	Chem. Absts. 1919, 251	Peat, sawdust, etc.	Dolomite plus MnO_2	Above 600°C.	

The table shows that the raw materials that have been employed for the preparation of vegetable decolorizing carbons are of the most diverse nature. In many cases they consist of carbohydrate materials or their derivatives, or of waste products obtained in the manufacture of them, viz., wood, sawdust, bagasse, paper; peat, lignite, coal. In other processes gums, wax residues, argol waste, are utilized. In still others bone-black is imitated by employing substances known to be high in nitrogen, like albuminoid substances, kitchen refuse, sulfite liquor, beet or cane molasses, certain forms of peat, etc.

Still greater is the diversity of impregnating materials used. In some cases none at all are employed. In others we find vapors or gases, like superheated steam, and chlorine. Then there are water soluble substances, as the alkalies and their carbonates, the chlorides of calcium, magnesium and zinc; also sulfuric acid. Some of the latter materials may be classed as dehydrating agents. Further there are mentioned acid soluble materials, like the alkaline earths and their carbonates, and oxide of iron; and finally substances little soluble or insoluble in water and most acids, as, for instance, clay. Many of the raw materials are already impregnated by nature or by some manufacturing process. To these belong culm (silicates), argol waste (salts of alkalis and calcium), sulfite waste liquor (calcium salts), molasses and bagasse (various ash constituents), filter press cake (calcium salts), shale residues (silicates).

The temperature of final carbonization also varies greatly, from that produced naturally upon mixing molasses and concentrated sulfuric acid, to as high as white heat. In many cases the impregnating substance is not afterwards removed, while in others it is taken out by an appropriate solvent. The object of all the processes evidently is to increase the porosity, and with it the adsorbing power of the carbon, but it will be readily seen that in spite of the large amount of data it is impossible to draw any conclusions concerning the connection between the methods of preparation of the carbons and their decolorizing power. This is due to the fact that many inventors do not express the decolorizing effect of their carbons in actual figures, as, for instance, by comparison with bone-black or another standard decolorizing substance, but often in non-committal and usually highly praising terms, such as very great, remarkable, etc. But even if the exact decolorizing effect were known, the details of the methods differ so much that they cannot be compared with one another.

Schneller[2] recognized the first of these difficulties, and proceeded to make some of the sawdust carbons mentioned in the above list in accordance with the directions of the inventors. He soon found that the relative quantity of impregnating substance used had a great influence on the decolorizing power of the carbon. Thus carbon prepared from two parts sawdust and one part calcium hydroxide was inferior to Norit; one from three parts sawdust and two parts lime about equal to Norit, and that from equal parts of the ingredients superior to it. The same observation was made in preparing carbons from sawdust and zinc chloride. Schneller also found that to obtain highly efficient carbons the final heating must be carried to bright red heat. Some of the inventors have even gone beyond this temperature, and, in fact, it was found by Coates[3] that ordinary wood charcoal from sawdust, the decolorizing power of which is only very slight, can be converted into a carbon equal to Norit by prolonged heating to 1200°C. in a covered crucible. This corroborates the results obtained by Lotz with lignite, in 1911.[4] It was also shown beyond doubt that in order to produce a highly efficient carbon, the impregnating substance must be removed after the final heating by some appropriate solvent. It is true that the impregnating substances themselves often have a slight decolorizing power, but this compares in no case with that of the carbon.

While Schneller was working on this subject, similar investigations were carried out in England by the sectional chemical committee of the Royal Society.[5] They found that it made little or no difference whether a coniferous or an angiospermous wood was used as raw material, but that it was necessary to impregnate it with some other substance. Lime and calcium acetate were used for this purpose, with little difference in the results. It was also proved again that the final carbonization had to be done at as high a temperature as possible, at least red heat, and that the impurities had finally to be removed, in this case by treating with hydrochloric acid.

When the senior author was called upon to investigate this subject further, the first thing which it seemed necessary to do was to establish the effect of different impregnating substances on the decolorizing power of the finished carbon under comparable conditions, using always the same raw material. For this purpose the sawdust of long leaf yellow pine *(Pinus palustris)* was used, and impregnated with different well-defined chemical compounds, bases as well as acids, halides, and other salts. Some other raw materials were also used for comparison, such as pure carbohydrates, as well as organic acids. In the first series of experiments a definite quantity of sawdust was mixed with a weighed amount of impregnating material, rubbed with water to a homogeneous paste, and the mixture heated in an open vessel, usually of iron, but in certain cases of porcelain, over a gas ring burner, until the mixture was perfectly dry, and always taking care that the organic matter did not ignite. In some cases the heating had to be carried out in a heavy iron retort provided with a condensing tube in order to keep the mixture from catching fire. The resulting product was ground, if necessary, and then in each case transferred to a receptacle made of a 5-inch nipple of 2-inch iron pipe, closed at both ends with iron caps. One of these caps was not screwed on absolutely tight, to give any gases evolved a chance to escape, without exposing the carbon to the action of the air. The final heating was done by placing this nipple in a muffle furnace at bright red heat, about 900°C., for a period of about two hours. In the case of some of the more volatile chlorides, like that of zinc, the duration of the heating was shortened. After cooling, the contents of the crucible were boiled out with appropriate solvents, caustic soda in some cases, hydrochloric acid or nitric acid in others. The excess of acid or alkali was again removed with water. The carbon was always finally washed with dilute hydrochloric acid and again with water. It was then dried at 105°C.

In order to get comparable results, the different impregnating compounds were used in chemically equivalent quantities. In the tests with basic hydroxides and carbonates, or with acids, the basis chosen was one part by weight of calcium oxide to one part of sawdust. The other compounds were taken in amounts corresponding to the quantity of calcium oxide employed; that is to say, for one gram molecule of CaO one gram molecule of compounds of bivalent elements, two gram molecules in the case of monovalent elements, two-thirds in that of trivalent ones, and so forth. With the salts of oxyacids, two series of tests were usually run, one where the salt was used in a quantity equivalent to the basic radicle, and another where the quantity was equivalent to the acid radicle. In the halide series the basis was two parts by weight of zinc chloride to one of sawdust. This is about twenty per cent less zinc chloride than corresponds to the quantity of calcium oxide used in the rest of the experiments. Therefore, while the different tests in the halide series are strictly comparable with each other, they bear no direct relation to the remaining ones. This has to be taken into consideration in interpreting the results compiled in Table III, where the tests of the halide series are therefore marked with an asterisk.

It will be noted that the preparation of the carbons was not carried out under perfect temperature control, but it was found that, whenever different samples of the same carbon were made to check the results, we usally obtained closely agreeing figures in determining the decolorizing power. This shows that any slight variations in the uncontrolled factors had no practical effect.

The decolorizing effect for sugar products of the carbons is determined in the following way. As a standard we employ a water solution containing 30 grams per liter of a final molasses, of which we keep a considerable stock on hand to have always the same basis. To 200 cc. of this molasses solution we add 5 grams of the carbon, bring just to the boiling point and filter through a folded filter. After the treated solution has cooled to room temperature the color is determined. We have used two different methods for the latter operation. At first Norit was used as the standard decolorizing carbon with which we compared all others. This was done by running an experiment in the same way as above described, but with 5 grams of Norit. The color of the solution obtained with the carbon under investigation was then compared in ordinary Nessler tubes with that produced by Norit, the decolorizing effect of which is called 100. The amounts of coloring matter left after treatment with the carbons are inversely proportional to the height of the columns of the same depth of color, and the decolorizing power of the carbon can be calculated from these results, as shown below. Unfortunately, sugar products usually contain several different coloring matters in varying proportions, and since the carbons show selective adsorption, the solutions produced by different carbons often differ greatly in tint. This difficulty could be obviated by using the carbon on some artificial standard coloring matter, but this did not seem advisable, because the ultimate object of our work was the preparation of carbons for the cane sugar industry, and for this very reason the selective adsorption property of the carbons had to be taken into consideration. As the method mentioned above gave only very approximate values, for the reason already indicated, some other process had to be found which yielded more accurate results. The ideal instrument for this kind of work is the spectrophotometer, but its practical use would have been too complicated for our purposes. The only serviceable apparatus which could be obtained at the time, owing to war conditions, was the Hess-Ives tint-photometer, and we decided on its use, although fully aware of its limitations. This instrument need not be described here, as an account of it has been given in Bulletin 165 of this station, and in more detail in a separate article.[6] In reading the decolorized solutions, the same volume of solution must, of course, always be used, and must be measured from a pipette or burette. It is run into a glass crystallizing dish, the same dish always being employed. This dish is placed over the right hand magnesia block on the support furnished by the manufacturers of the instrument, and in order to compensate for the light absorption due to the glass bottom of the dish and to the water in the solution, another similar dish is to be placed above the other magnesia block and filled with sufficient water to counterbalance the effect of the water in the solution on the other side. Then readings are made and recorded, for the three colors as well as for the sum of all three. The degrees read on the instrument are not in direct proportion to the color concentration, and we therefore constructed curves showing the degrees of the instrument corresponding with different concentrations of coloring matter, ascertained by actually diluting the original molasses solution to different percentage concentrations.

In order to give the reader an idea of the comparative decolorizing power of the carbons prepared by us, we have assembled below the figures for the decolorizing effect of some of the better known decolorizing agents. Readings through the red glass are reported as green, those under the green glass as red, and those under the blue glass as yellow; that is, always in terms of the complementary colors. The figures represent percentages of color removed by each carbon.

TABLE II.

		% Color Removed			
		Green	Red	Yellow	Total
No.	Carbon Used				
140	Bone-black granules of 1 mm. diam.	0	0	0	0
139	Bone-black purified, fine powder	81	61	40	66
141	Carbon Black "G"	71	64	55	65
143	Francesconi Black	8	10	15	9
144	Norit	84	81	80	81
182	Norit, washed with acid	92	89	87	89

It is readily seen that under the conditions of our standard method the decolorizing power of ordinary bone-black is so slight that it can not be measured. Norit, on the other hand, removes more total coloring matter than any of the other materials.

Table III, below, contains a list of all the sawdust carbons made by us, arranged according to their decolorizing power. In this table we have added a further column, giving the "efficiency" of each carbon in terms of Norit. It will be shown in the discussoin of the results that this carbon serves the purpose excellently. Norit, under the special conditions of our method, removes 81% of coloring matter. Another carbon may remove, say, 96%. This means that the second carbon takes out only 15% more than Norit, calculated on the quantity present in the original solution. But in using decolorizing agents the quantity of coloring matter *remaining* in the treated solution is of much greater importance than the quantity *removed* by the carbon. From this standpoint the second carbon cited above is not 15% more efficient than Norit, but 19/4, or 4.75 times as efficient as Norit, since it leaves only 4% of coloring matter in the liquor, as compared to the 19% left by Norit. All the efficiency figures given in Table III are calculated on the basis of 100 for Norit.

Dr. Bancroft has justly remarked about this method of expressing the efficiency of the carbons, that it gives an exaggerated idea of the actual adsorptive effect. This is perfectly true, as far as the properties of the carbon itself are concerned. There is probably very little difference in the structure of two carbons which remove, say, 96% and 99% of coloring matter respectively under the conditions of our test. But from the standpoint of the practical sugar manufacturer the matter appears in an entirely different light. The liquor treated with the first carbon is still distinctly yellow in a two inch layer, while that decolorized with the second is practically water white, containing only one-fourth as much coloring matter as the first.

The relative success attained in finally purifying the carbons with either acid or alkali, followed with washing by water, acid, and again water, is shown by the ash figures in the last column of the table. In most cases the ash is so low that its removal would not appreciably affect the decolorizing power of the carbon. But there are some carbons, like Nos. 46 and 47, where insoluble oxides were formed, Nos. 43 and 11, where barium sulfate was evidently obtained, and some others, in all of which account must be taken of the fact that the decolorizing power of the purified carbon would be higher than that actually found.

TABLE III.

No.	Impregnating Substance Used:	% Color Removed				Efficiency of Carbon	Ash in Carbon
		Green	Red	Yellow	Total		
41	1 mol. Sodium carbonate	0	0	0	0	19	18.84
155	1/3 mol. Sulfuric acid	0	2.5	15	2	19.4	1.87
77	*2 mols. Potassium chloride	10	0	0	5	20	3.54
8	*2 mols. Sodium chloride	20	10	15	15	22	2.80
57	1/3 mol. Sodium sulfate	23	20	15	20	24	2.87
54	2/5 mol. di-Sodium phosphate	28	20	15	22	24	8.70
123	*2 mols. Sodium bromide	26	25	15	25	25	1.98
44	2 mols. Sodium hydroxide	28	20	5	25	25	9.83
124	*2 mols. Sodium iodide	34	29	20	32	28	4.32
55	2/3 mol. Boric acid	38	37	35	37	30	0.83
58	1/8 mol. Sodium tetrasilicate	43	35	25	39	31	16.01
45	2 mols. Potassium hydroxide	44	40	35	41	32	0.83
154	2/5 mol. o-Phosphoric acid	45	37.5	40	41	32	6.19
56	1/6 mol. Sodium tetraborate	43	37	40	41	32	1.26
18	*1 mol. Lead chloride	45	35	25	41	32	14.32
78	1 mol. Lithium carbonate	45	40	40	43	33	5.53
62	1/9 mol. Aluminum sulfate	45	37	27	42	33	7.24
53	2/5 mol. di-Ammonium phosphate	55	43	40	47	36	3.12
61	1/3 mol. Ammonium sulfate	55	45	40	48	37	1.25
26	*2 mols. Ammonium chloride	63	45	25	51	39	5.07
50	1/2 mol. Silicon oxide	59	46	45	51	39	24.06
59	1/9 mol. Calcium hexaborate (Colemanite)	55	50	45	51	39	20.68

153	1/2 mol. Calcium hexaborate (Colemanite)	63	56	45	57	44	47.44
21	*2/3 mol. Ferric chloride	63	54	50	58	45	2.90
181	1 mol. Calcium metaphosphate	67	53	50	59	46	51.96
31	*1 mol. Calcium chloride, plus excess ammonium chloride	65	60	45	60	48	17.66
148	1 mol. Sodium sulphate	66	62	60	63	51	2.13
112	1/2 mol. Calcium monosilicate	*	*	*	65	54	12.04
60	1/3 mol. Calcium sulfate	74	62	61	66	56	3.45
27	*1 mol. Cupric chloride	75	62	57	66	56	5.60
149	1 mol. di-Sodium phosphate	77	74	70	73	70	...
51	*2 mols. Lithium chloride	86	74	67	76	79	5.80
11	*1 mol. Barium chloride	83	76	72	77	83	32.25
34	*2/3 mol. Aluminum chloride, plus excess ammonium chloride	86	78	70	78	86	39.18
12	*1 mol. Manganous chloride	86	78	74	79	90	12.63
161	1 mol. Calcium carbonate	84	82	74	81.5	103	6.75
156	1/5 mol. Phosphorus pentoxide	86	85	76	83	112	7.59
49	*1 mol. Cadmium chloride	90	83	88	83	112	2.26
150	1 mol. Sodium tetraborate	87	85	80.5	84	119	2.57
22	*1 mol. Ferrous chloride	89	86	83	85	127	2.33
25	*1 mol. Nickel chloride	87	85	84	85	127	12.73
68	1/5 mol. Calcium o-Phosphate tribasic	89	86	81	85	127	1.82
47	2/3 mol. Chromium hydroxide	96	88	89	88	158	75.61
151	1 mol. Calcium sulfate	91	89	88.5	89	173	3.60
152	1/3 mol. Calcium o-Phosphate tribasic	92	89	88	89	173	2.05
46	2/3 mol. Aluminum hydroxide	95	91.5	90	92	238	24.34
66	*1 mol. Zinc iodide	95	93	91.5	92.5	253	0.53
43	1 mol. Barium hydroxide	97	94.5	91	93.5	292	48.30
16	*1 mol. Stannous chloride	97	92.5	96.5	93.5	292	1.37
42	1 mol. Magnesium hydroxide	98.5	96.5	93.5	95.5	422	4.93
71	*1 mol. Zinc bromide	98	95.5	94	95.5	422	1.23
40	1 mol. Calcium oxide	99.5	96.5	94	95.5	422	12.38
15	*1 mol. Zinc chloride	98	96.5	95	96	475	9.63
20	*1 mol. Magnesium chloride, plus excess ammonium chloride	100	99.4	99.4	99.4	3200	1.75

The results confirm our previous observation that the different carbons remove the coloring matters of sugar products in different proportions, and that therefore the tints of the treated solutions are not alike, even though the depths of color might be equal. This is strikingly shown by comparing carbons No. 45, 154, 56, and 18, and again carbons No. 26, 50, and 59. In each of these groups the total decolorizing power of the carbons is found to be the same, but they differ greatly in the proportions of each component color removed.

It is further noted that the carbons generally take out a greater proportion of green than of red, and again more red than yellow; but the total effect is usually in about the same ratio as for red, although there are many exceptions.

It is readily seen that the nature of the impregnating substance used has a pronounced effect on the decolorizing power of the resulting carbons. Some of these are extremely poor, taking out little or no color, while others remove almost all of it. The differences in decolorizing power become even more apparent upon examining the efficiency figures given in the table. They show that the poorest carbons are about one-fifth as efficient as Norit, while Norit is only about one-fifth as efficient as our best carbon but one. This last one, No. 20, under the conditions of the test, removes the coloring matter almost completely.

In the case of the last seven carbons shown in the table, the tintometer readings are so small, especially under the red glass, and the percentages of color removed are so close together that the efficiency figures are only approximately correct. It becomes important therefore to check up these figures by those obtained in ordinary colorimetric measurements. Such a comparison is given in the following table:

TABLE IV.

No.	Efficiency by Tintometer	Efficiency by Colorimeter
43	292	224
16	292	350
42	422	306
71	422	300
40	422	320
15	475	350
20	3200	1500

It is seen that the efficiency figures according to the colorimetric method are lower than those obtained by the tintometric one, but with the exception of carbon No. 16 they are in about the same serial order.

Studying the table from the standpoint of possible connections between the nature of the impregnating substances used and the decolorizing power of the resulting carbons, we find that both physical and chemical factors seem to play a part. In a general way it appears that those materials which are either solid or gaseous at the temperature of the final heating tend to produce good carbons (for instance, Nos. 68 to 20, all at the bottom of the list), while those that are liquid tend to produce poor ones (for instance, 41, 77, 8, 125, 44, 124, 55, 45, all near the top of the list). From the chemical point of view it would seem that those compounds which are known to attack cellulose most vigorously tend to produce good carbons, while the more indifferent substances furnish poor ones. But the physical factors are evidently more important, because else the hydroxides and carbonates of the alkalis should furnish superior carbons, which, in fact, they do not.

However, there are many apparent exceptions to these rules, and it will therefore be necessary to consider the different carbons in greater detail.

Impregnation with chlorides. It has already been mentioned that the chloride carbons were not made on exactly the same basis as the others, and we shall therefore consider them seprately before taking up the others.

As the state of aggregation of the impregnating material at the temperature of final heating seems to be an important factor in developing decolorizing power, we have arranged a table giving, besides the efficiency of the carbon, also the melting and boiling points of the different chlorides. The chemical energy of the impregnating materials is more difficult to state in figures, because there is no standard measure of chemical affinity as yet. However, the chemical effect upon carbohydrates of at least some of the chlorides is undoubtedly connected with their dehydrating power, or, in other words, with their heat of hydration under the conditions of the tests. The magnitude of this factor is not known directly, since it has been determined only for a few chlorides, and only under certain conditions different from those in our experiments. However, as the amount of water to be removed from the organic matter of the sawdust is always the same, the heat of hydration would in each case be equal to the heat of solution of the particular chloride, less the heat of dilution between the concentration obtaining in the test and the concentration at which the heat of solution is determined. In the few cases where the heat of solution and heat of dilution have been determined, the latter is generally small as compared to the former, and there is a certain parallelism between the heat of hydration and the heat of solution. Since the heat of solution has been ascertained for all of the chlorides used by us, we are including them in the following table as an approximate measure of the heat of hydration or dehydrating power of the chlorides.

TABLE V.

No.	Chloride Used	Melting Point °C.	Boiling Point °C.	State of Aggregation at Red Heat	Heat of Solution cal.	Efficiency of carbon	% Ash in carbon
			of Chloride Used				
77	$2\,\mathrm{KCl}$	772	1500	Liquid	−4440	20	3.54
8	$2\,\mathrm{NaCl}$	804	1490	Liquid	−1180	22	2.80
18	$\mathrm{PbCl_2}$	500	950	Liquid to gas	−6800	32	14.32
26	$2\,\mathrm{NH_4Cl}$	—	Dec.	Remvd.	−3880	39	5.07
21	$\frac{2}{3}\,\mathrm{FeCl_3}$	—	280	Remvd.	63360	45	2.90
31	$\mathrm{CaCl_2}$, plus $\mathrm{NH_4Cl}$	774	White Heat	Liquid	17400	48	17.66
27	$\mathrm{CuCl_2}$	498	Dec. ab. 950	Liquid	11080	56	5.60
51	$2\,\mathrm{LiCl}$	602	1360	Liquid	8840	79	5.80
11	$\mathrm{BaCl_2}$	955	White heat	Solid to liquid	2070	83	32.25
34	$\frac{2}{3}\,\mathrm{AlCl_3}$, plus excess $\mathrm{NH_4Cl}$	(190)	183	Remvd.	153690	86	39.18
12	$\mathrm{MnCl_2}$	650	Red ht.	Gas	16010	90	12.63
49	$\mathrm{CdCl_2}$	568	900	Gas	3010	112	2.26
22	$\mathrm{FeCl_2}$	Red heat	Subl. red heat	Gas	17900	127	2.33
25	$\mathrm{NiCl_2}$	—	Subl. red ht.	Gas	19170	127	12.73
16	$\mathrm{SnCl_2}$	249	626	Gas	5730	292	1.37
15	$\mathrm{ZnCl_2}$	262	730	Gas	15630	475	9.63
20	$\mathrm{MgCl_2}$, plus excess $\mathrm{NH_4Cl}$	708	Red ht.	Gas	35920	3200	1.75

Unfortunately, the volatilization points of some of the chlorides used are not known exactly, and a further difficulty arises from the fact that we do not know what chemical changes the chlorides undergo when gradually heated with sawdust to a final temperature of about 900°, which was about the average temperature used in the final heating.

It will be noted that in the cases of some chlorides which are readily decomposed by water at high temperatures, we added an excess of ammonium chloride to avoid such decomposition. While this method works quite well with the chlorides of calcium and magnesium, it is of very little effect with aluminum chloride and also with ferric chloride, in which latter case the treatment was therefore omitted. The effect of such additions as ammonium chloride was studied a little more closely, as will be seen below. It stands to reason that a large quantity of aluminum chloride and also of ferric chloride is lost by volatilization during the preliminary heating, and that there is none left as such during the final heating. The same is true for the ammonium chloride, used alone. All these cases are indicated in the table by the caption "Removed," in the column headed "State of Aggregation."

Taking all these factors into consideration and leaving out the cases in which the impregnating substances are no more present during the final heating, the results show that those chlorides which are liquid at that temperature yield poor carbons, while those that are volatilized give good ones. Barium chloride which remains solid at the temperature used, but is not far from the liquefaction point, stands exactly between the other two groups. This proves what we had concluded before, namely, that the state of aggregation of the impregnating substance at the temperature of the final heating is evidently the most important factor to be considered. This conclusion is further strengthened by the fact that those chlorides which volatilize readily, like those of tin and zinc, tend to give better carbons than those which do not volatilize so easily, like those of cadmium, bivalent iron and nickel. But here the chemical energy of the chlorides has to be considered also. It is noticed that all the chlorides with negative heat of solution give very poor carbons, all found at the top of the list, even though one of them—lead chloride—is quite close to the boiling point at the temperature used. With those chlorides that remain liquid, it does not seem to make much difference whether the heat of solution is low or high. But with the others the heat of solution is an important factor, as shown by carbon No. 20, which is by far the most effective of all.

Dr. Bancroft is of the opinion that this connection between the decolorizing power of the carbon and the heat of solution is only a coincidence. This in itself is evidently true, as we have explained above, but it was also shown there that the heat of solution of the various chlorides is the only figure found in the literature which has any relation whatever with the dehydrating power. Investigations in the thermochemical field will have to show whether the connection between the decolorizing power of the carbons and the dehydrating power of the chlorides is accidental or real. It may be mentioned here that Lamb, Wilson, and Chaney[16] report as follows, in speaking of the preparation of gas masks: "It has been found, however, that by certain chemical processes, such as heating the wood up with strong sulfuric acid, hydrochloric acid and zinc chloride, etc., it is possible to remove practically all of the hydrogen and oxygen as *water,* leaving behind a very dense carbon which, since there is practically no hydrocarbon present, needs relatively little treatment (except washing out the chemicals) to make a satisfactory charcoal."

Summing up, the tests with chlorides have shown that chlorides which volatilize or are largely decomposed at the temperature of the preliminary heating, tend to give poor carbons. Chlorides other than those in the former class yield poor carbons if they are in the liquid state at the temperature of the final heating, and good carbons if they are gaseous. (We shall see later that impregnating substances that remain solid also tend to give good carbons; see also carbon No. 11, which has a high ash, and is therefore not as efficient as it would be in the ash-free state.) Other conditions being equal, the chlorides which near the temperature of final heating are more volatile, give better carbons than the less volatile ones. Chlorides having negative heat of solution yield poor carbons. Those having positive heat of solution give good carbons, provided that the chloride is not liquid at the temperature of the final heating. Other conditions being equal, the decolorizing power of the carbon increases directly as the heat of solution of the chloride employed in its preparation.

The effect of preventing the decomposition of those chlorides which are easily affected by water at high temperatures may be seen from the results of the following tests:

TABLE VI.

No.	Chloride Used:	Efficiency of Carbon	Percent Ash in Carbon
20	$MgCl_2$, plus excess NH_4Cl	3200	1.75
19	$MgCl_2$, without NH_4Cl	59	1.64
31	$CaCl_2$, plus excess NH_4Cl	48	17.66
10	$CaCl_2$, without NH_4Cl	44	4.08
34	$AlCl_3$, plus excess NH_4Cl	86	39.18
36	$AlCl_3$, plus excess NaCl	51	21.51
35	$AlCl_3$, without addition	45	52.67

In the case of magnesium chloride the addition of ammonium chloride evidently gives very striking results, but the effect is also quite noticeable in the other two series, especially if the ash content of the carbons is taken into consideration.

Some further tests were also made in which we compared the effect of the bromides and iodides of two metals with that of their chlorides. The results were as follows:

TABLE VII.

No.	Halide Used:	Melting Point	Boiling Point	Heat of Solution	Efficieny of Carbon	% Ash in Carbon
15	$ZnCl_2$	262	730	15,630	475	9.63
71	$ZnBr_2$	394	650	15,030	422	1.23
66	ZnI_2	446	624	11,310	253	0.53
8	NaCl	804	1490	—1,180	22	2.80
123	NaBr	756	1455	—190	25	1.98
124	NaI	653	1350	1,220	28	4.32

The halides evidently follow the general rules found for the chlorides alone. The three zinc halides are all gaseous at the temperature of the final heating, and their effect decreases directly with their heat of solution. The sodium halides are all liquid, far from the volatilization point, and their effect also changes in the same direction as their heat of solution.

Impregnation with oxides, etc. We shall next take up the group of carbons prepared by impregnation with oxides, including their acid and basic derivatives, and also with carbonates, leaving the remaining salts to be considered separately. Only a limited number of oxides could be tried, because the oxides of most of the heavy metals are reduced by carbon, often with the formation of carbides, and they would therefore be useless for our purpose.

The results obtained in this series are much more difficult to interpret, because, unlike the chlorides, a number of the impregnating substances used here undergo important chemical changes during the process of carbonization. Thus, for instance, the sodium hydroxide used in one of the tests combines with the carbon dioxide evolved and forms sodium carbonate, which at the temperature of final heating is only slightly, if at all, attacked by the carbon. For this reason we have made an attempt to ascertain the changes undergone by the impregnating substances during the process, but it is impossible to do this with any degree of accuracy, since the reactions which take place under the circumstances have not been studied to any extent.

The heat of solution is not as good a criterion here as in the case of the chlorides. The heat of solution of such substances as the hydroxides of aluminum or silicon is in itself negative, its numerical value being equal to the heat of precipitation, but under the conditions of the tests this latter factor may be left out of consideration. The chemical energy of the impregnating substances may more properly be judged in these cases from the position of the corresponding elements in the periodic system, and also from what is known about the energy with which the compounds attack carbohydrates. From this standpoint the oxides, etc., of the alkalis and alkaline earth metals would rank first, followed by magnesia, alumina and chromium hydroxide. Among the compounds of an acid nature the proper sequence would be phosphorus pentoxide, sulfuric acid, phosphoric acid, boric acid, and silica.

While the melting points of the impregnating compounds used are in mosts cases known approximately, the boiling points of a number of them have not been ascertained. All of these factors have to be considered in interpreting the following table:

TABLE VIII.

No.	Material Used	Heat of Solution cal.	Material Formed	Heat of Solution cal.	Melting Point of Material formed, °C.	Efficiency of Carbon	% Ash in Carbon
41	Na_2CO_3	5640	Na_2CO_3	5640	849	19	18.84
155	H_2SO_4	11470	SO_2 Removed	1500	−76	19.4	1.87
44	NaOH	9940	Na_2CO_3	5640	849	25	9.83
55	BO_3H_3	−10790	B_2O_3	7300	577	30	0.83
45	KOH	13290	K_2CO_3	6490	909	32	0.83
154	H_3PO_4	5350	?	?	?	32	6.19
78	Li_2CO_3	—	?	?	?	33	5.53
50	SiO_2	—	SiO_2	—	1600	39	24.06
161	$CaCO_3$	—	CaO	18330	2570	103	6.75
156	P_2O_5	53600	?	?	?	112	7.59
47	CrO_3H_3	—	Cr_2O_3	—	1990	158	75.61
46	AlO_3H_3	—	Al_2O_3	—	2050	238	24.34
43	BaO_2H_2	12260	BaO	34520	White ht.	292	48.30
42	MgO_2H_2	—	MgO	9400	2800	422	4.93
40	CaO	18330	CaO	18330	2570	422	12.38

As we had previously observed in the case of chlorides, those substances which volatilize during the preliminary heating give poor carbons. This is shown in No. 155; the sulfuric acid is largely reduced to sulfur dioxide, which escapes, and any excess of sulfuric acid also volatilizes much below red heat. For this reason the acid gives a poor carbon, in spite of its high heat of solution.

It is further noted that in general those substances which are liquid at the temperature of final heating (41, 44, 55, 45) also yield poor carbons, while those that are solid give good ones. Those materials which are known to attack carbohydrates most vigorously tend to give efficient carbons, unless the compounds formed from them are liquid at the temperature of final heating. This point is especially well shown by comparing, for instance, Nos. 43, 42, and 40 with 44 and 45. Phosphorus pentoxide, with its high heat of solution, gives a much better carbon than orthophosphoric acid, and, for the same reason, calcium oxide a much better one than calcium carbonate. Silica, although being infusible, yields a rather poor carbon, while alumina gives a good one. This is probably partly due to the fact that silica is chemically almost inert, while alumina has slightly basic properties. But a more potent reason is found in the very finely divided state of the aluminum hydroxide used which tends to make the carbon more porous. We shall see later that if silica is finely divided and intimately mixed with organic material, as happens in certain plant materials, the carbon obtained is much better than that prepared in the above test. Barium hydroxide also would have yielded a much better carbon, if it had been possible to reduce the ash further. The same is true for chromium hydroxide. Lithium carbonate gives a rather poor carbon, but better than sodium hydroxide and sodium carbonate. It is probable that some of the carbonate is converted by the carbon into infusible oxide.

Impregnation with salts. The last group of impregnating substances comprises a number of different salts. Several of these were used in two different quantities, one corresponding to the acid radicle of the salt, and the other corresponding to the basic one. In this group it is even more difficult to know what chemical changes actually take place during the process of carbonization. It may be expected that in quite a few of the processes the carbon formed acts on the substances used, giving rise to other compounds, mixtures of which with the original compound may have low melting points. The chemical action of all these salts on carbohydrates is only feeble, and we would therefore expect them to yield poor or only moderately good carbons. This expectation is borne out by the facts, as may be seen from the following table, in which only the relative quantities of the salt used, and the decolorizing power of the resulting carbons are given, because physical and chemical data bearing on the problem are either unknown or of little value for our purpose.

TABLE IX.

No.	Material Used:	Efficiency of Carbon	% Ash in Carbon
148	1 mol. Sodium sulfate	51	2.13
57	1/3 mol. Sodium sulfate	24	2.87
149	1 mol. di-Sodium phosphate	70	
54	2/5 mol. di-Sodium phosphate	24	8.70
58	1/8 mol. Sodium tetrasilicate	31	16.01
150	1 mol. Sodium tetraborate	119	2.57
56	1/6 mol. Sodium tetraborate	32	1.26
62	1/9 mol. Aluminum sulfate	33	7.24
53	2/5 mol. di-Ammonium phosphate	36	3.12
61	1/3 mol. Ammonium sulfate	37	1.25
153	1/2 mol. Calcium hexaborate (Colemanite)	44	47.44
59	1/9 mol. Calcium hexaborate (Colemanite)	39	20.68
112	1/2 mol. Calcium monosilicate	54	12.04
151	1 mol. Calcium sulfate	173	3.60
60	1/3 mol. Calcium sulfate	56	3.45
152	1/3 mol. tri-Calcium phosphate	173	2.05
68	1/5 mol. tri-Calcium phosphate	127	1.82
181	1 mol. Calcium metaphosphate	46	51.96

Most of the carbons are comparatively poor, as was to be expected for reasons stated above. Those made with the aid of calcium salts are generally better than those prepared with the corresponding sodium salts; this is probably due to the greater fusibility of the sodium compounds formed in the process as compared to that of the calcium compounds. Comparing the effects of different salts of the same base (60, 68, 59, and 112; 57, 54, and 58; the other quantities are not comparable), we find that calcium phosphate is the most effective, followed by sulfate, silicate and borate. The latter two probably melt more readily than the sulfate, or, rather, the sulfide formed from it, and the phosphate is still less fusible. In the case of the corresponding sodium salts we find very little difference in the effect of the different salts. The fact that calcium phosphate is the most effective of all the salts used under comparative conditions, is very interesting, because it forms the skeleton of bone-black, which has dominated the refining industry for over one hundred years. Calcium metaphosphate gives a poor carbon, largely owing to the fact that it is difficult to remove by the ordinary solvents, and the carbon obtained is therefore high in ash.

The results also show again that the efficiency of the carbon increases with the proportion of impregnating substances per unit of raw material. Borax, used in the larger quantity, gave a relatively good carbon, because, like aluminum hydroxide, it is very finely divided, and this aids mechanically in developing the porosity of the carbon. Aluminum sulfate, on the other hand, gave a poor carbon, as it is quite dense, and the quantity of alumina actually present during the final heating is quite small, only 1/9 molecule of sulfate being used in the test—that is, in proportion to the acid radicle. The two ammonium salts tried gave somewhat better carbons than the corresponding acids alone. Here the evolution of gaseous ammonia at high temperatures probably helped in increasing the porosity of the carbon.

Instead of using the salts ready formed, they might also be added by first treating the raw material with the corresponding base and then with the acid, or vice versa. Lotz[7] found that in this way much better carbons are obtained than by using the salt itself. This stands to reason, because in this case either the acid or the base can exert its chemical action on the organic matter. In one experiment we first added phosphoric acid to the sawdust and then neutralized with lime. The resulting carbon showed an efficiency of 270, which is over twice as high as that of the carbon prepared with an equivalent quantity of ready formed calcium phosphate.

Tests with other raw materials. A few experiments were next made to study the effect of using pure carbohydrates instead of wood by impregnating these materials with equal quantities of calcium hydroxide. The results were as follows, determined by ordinary colorimetric methods:

TABLE X.

No.	Carbohydrate Used:	Efficiency
23	Paper cellulose	170
14	Sucrose	.240
29	Dextrose	270
24	Starch	293

It would appear from these figures that the vigor with which the lime attacks these substances has something to do with the efficiency of the carbon made from them. Dextrose is very readily attacked and gives an excellent carbon, then follows sucrose, and finally cellulose. Starch gives a still better carbon than the others, probably owing to its fine division.

Some carbons were also made from the sodium and calcium salts of acetic, tartaric and citric acids. Naturally only very small quantities of carbon were obtained in these cases, and no regular color determinations could be made. But in each case the calcium salts gave very active carbons, several times better than Norit, while the sodium salts yielded very poor carbons, greatly inferior to Norit. This is in strict agreement with what we had found before.

Effect of nitrogen. In all the work done so far no attention was paid to a possible effect of nitrogen on the decolorizing power of the carbons. Various investigators have come to the conclusion that the effect of bone-black and other carbons is due to complex C—N compounds contained in them. Although this question comes more properly under the category of properties of carbons, it must also be taken into consideration when investigating their methods of preparation. It has recently been shown that carbon combines directly with nitrogen at very low temperatures, and it is quite possible that in the methods of preparation used by us nitrogen is taken up chemically, either from the atmosphere or from the ammonium salts added in several of the tests. Sawdust itself, of course, contains some nitrogen, but the fact that pure sucrose and dextrose impregnated with lime give very good carbons would speak against the nitrogen theory, unless atmospheric nitrogen were taken up. This question can be studied better by an analysis of the different carbons at the time when we take up the question of their properties in general. It must be considered, however, that the addition of nitrogenous substances might in some way affect the formation of the carbon without nitrogen actually appearing in the final carbon.

In our experiments on carbons from Pacific Coast kelp, which will be fully described on pages 35-40, we found that by the use of our carbonization method a very much better carbon was obtained than we would have expected of a material which consists practically of organic matter impregnated with potassium chloride. When the carbonization product after the final heating was treated with hydrochloric acid, we often noticed the formation of prussian blue, a proof that cyanogen compounds had been formed in the process. We therefore made an experiment in which sawdust was treated with potassium chloride and albumen, leaving out the albumen in another parallel test. One hundred and forty grams of sawdust were mixed with 60 grams of potassium chloride, and in the other experiment enough albumen was added to bring the nitrogen content of the mixture to 1%, which is about the figure found in certain kelps. The carbon made without the addition of albumen removed 37.5% of coloring matter, which corresponds to an efficiency of 30, while that made with albumen removed 54.5% of coloring matter, giving an efficiency of 42, considerably higher than the other. Absolutely speaking, it is still quite low, but it must be considered that in an artificial mixture as was used in the experiment there is no such intimate contact between the nitrogen and the rest of the organic matter as there is in a material like kelp, or, for instance, cottonseed meal. This latter material, which has only a low ash content but is high in nitrogen, gives a carbon of an efficiency of 114, as against 19 for ordinary sawdust carbon.

CONCLUSIONS.

1. *Effect of chemical character of raw material.* Only materials consisting largely or exclusively of carbohydrates, and also some organic acids, have been investigated from this point of view. It was found that, as long as the raw material contains little or no nitrogen, its chemical character is of little importance. But organic nitrogen added to or present in the raw material increases the decolorizing power of the resulting carbon.

2. *Effect of state of aggregation of impregnating material at temperature of final heating.* Those materials which are either solid or gaseous at the temperature of final heating produce effective carbons, while those that are liquid give poor ones.

3. *Effect of chemical nature of impregnating substance.* Those compounds which attack carbohydrates and similar materials most vigorously, either on account of their dehydrating effect or through other chemical energy, give effective carbons, provided that they do not give rise to compounds which are liquid at the temperature of final heating. Other conditions being equal, those having the highest chemical energy produce the best carbons.

4. *Effect of fineness of division of both raw material and impregnating substance.* If raw material or impregnating substance, or both, are finely divided, they give a better carbon than if they are in a coarser state.

5. *Effect of the relative quantity of impregnating substance used.* The decolorizing power of the carbon increases directly with the quantity of impregnating substance used per unit of raw material.

6. *Effect of method of preliminary carbonization.* In our tests better carbons were generally produced by effecting the first heating in such a way that the fumes evolved could freely escape, care being taken, however, that the material did not ignite. Carrying out the first heating in a closed vessel had a depressing effect.

7. *Effect of the temperature of final heating.* Within the limits of the temperatures investigated the decolorizing power of the carbons increased with the temperature employed in the final heating. Further tests along this line will be found on pages 38-39.

II. PREPARATION OF DECOLORIZING CARBONS FROM MATERIALS IMPREGNATED BY NATURE OR BY SOME MANUFACTURING PROCESS.

The experiments described so far were made as much as possible under controlled conditions, and they enabled us to find a way for preparing a carbon of extremely high decolorizing power. The results obtained furnished us a basis for a search after cheap raw materials that might be confidently expected to yield effective carbons for production on a commercial scale. With this object in view we prepared carbons from a number of materials found in nature or obtained as by-products.

A. DECOLORIZING CARBONS FROM PLANT MATERIALS PROPER.

Most plant materials generally met with contain little ash, and this ash consists largely of alkali salts which are readily fusible. For this reason ordinary plant charcoals are usually low in decolorizing power. But there exists a small number of plants or parts of plants which not only have a high ash content, but the ash of which consists largely of infusible material, generally either lime and magnesia salts, or silica. Some few plants have the further advantage that they are high in nitrogen.

It would be expected that plant materials high in ash would give better carbons than artificial mixtures prepared from sawdust and the respective impregnating substances in the same proportions, because the mixture in the plant material is much more intimate than can be obtained by mechanical means. This expectation is borne out by the facts; for instance, in the case of the rice hull carbon mentioned below.

From chemical analyses made by previous investigators it appeared that promising materials might be found among the cryptogams, as well as among the phanerogams.

Of the former might be mentioned some thallophytes, like Sargassum, Chara, Ulva and Chondrus crispus (high in ash, lime and nitrogen), and a number of others which are known for their lime incrustations, as, for instance, Cladophora, Oedogonium, Halimeda, Acetabularia, Corallina. Among the mosses the most noteworthy is Pellia epiphylla (high in ash with much silica), and among the higher cryptogams Equisetum (comparatively high in ash, rich in silica).

The most promising phanerogams are Oryza sativa (leaves and hulls high in ash, which consists almost entirely of silica); Hordeum vulgare (awns very high in ash, with high silica content); Celtis and Lithospermum (seeds high in ash and lime); certain plants with lime incrustations, like Ceratophyllum, Potamogeton, Elodea, Saxifraga, or with silica incrustations, like Podostemon, Hirtella, Licania.

We have been able to study only a few of the plant materials named above, and the results are given in the following table. The first column gives the name of the material, the second the total ash, the third the sum of infusible oxides (lime, magnesia, iron oxide and alumina), the fourth the silica, the fifth the nitrogen, the sixth the chemicals with which the carbon was purified, the seventh the ash in the carbon, and the last its efficiency.

Table XI.

Name of Material	Ash %	Lime, etc., %	Silica %	Nitrogen, %	Treatment	Ash in Carbon %	Efficiency of Carbon
Andropogon muricatus, leaves*	11.0	Ash quite infusible			NaOH HCl		63
Celtis, seeds	37.04	21.00	4.07	1.63	HCl	31.61	46
Ceratophyllum	21.03	3.59	4.62	3.99	HCl	18.13	32
Chondrus crispus	24.76	2.89	2.62	2.47	NaOH, HCl	3.63	86
Equisetum robustum and levigatum*	20.0	Ash little fusible			NaOH HCl		72
Licania pyrifolia, bark	9.73	8.78	0.42	0.43	NaOH HCl	6.65	29
Macrocystis pyrifera	35.9	5.90		1.59	HCl	5.86	280
Opuntia arborescens, stems	4.75	2.20	1.26	0.47	HCl	9.29	25
Opuntia dulcis, slabs	19.59	10.44	3.49	0.75	HCl	12.20	24
Oryza sativa, leaves	26.34	very low	very high	0.40	NaOH HCl	48.34	66
Oryza sativa, hulls	21.75	very low	very high	0.42	NaOH HCl	12.63	66
Pinus palustris, wood	0.47	Not detd	Not detd	0.18	HCl	1.90	19
Pteris	7.69	0.88	4.25	1.63	NaOH HCl	5.32	41
Saccharum officinarum, leaves*	10.0	Ash quite infusible			NaOH HCl		63
Sargassum	35.16	7.71	7.39	1.76	NaOH HCl	23.24	160
Soja hispida, stems	4.90	1.74	0.19	1.71	HCl	7.16	27
Ulva	24.85	7.98	5.08	1.88	NaOH HCl	21.10	73
Unknown Mexican fodder plant	15.26	11.57	0.18	0.57	HCl	6.02	70

*These data taken from Dr. Schneller's notes.

The results confirm in a general way those obtained before. Leaving out the carbon from Celtis seeds, the plant with the highest ash content, Macrocystis pyrifera, gave the best carbon, while that with the lowest, Pinus palustris, gave the poorest. The tests with Macrocystis will be taken up in detail below. Sargassum, with the next highest ash content, fairly high infusible ash constituents, and high nitrogen, gave the second best carbon. If the ash in the carbon could have been reduced further, the efficiency of the carbon would no doubt have been higher. The third best carbon was prepared from Chondrus crispus; here the total ash is still high, and, while the infusible ash constituents are low, the nitrogen is very high. Then follows the carbon from Ulva, which plant has about the same ash content as Chondrus crispus, with more infusible ash, but less nitrogen; the decolorizing power of the carbon would be higher if the ash could be further reduced. For the same reason the Celtis seeds give a much poorer carbon than would be expected from their composition. The ash of the carbon consists here evidently largely of meta- and pyro-phosphates which dissolve with difficulty. Passing now to the materials which have a lower total ash content than the previous ones, but the ash of which consists largely or almost entirely of silica (the leaves of sweet grass, sugar cane and rice; horsetail and rice hulls), we find that all of these give comparatively good carbons, ranging in efficiency from 63 to 72. It may be mentioned here that Dr. Schneller obtained from barley awns, which belong in the same class of plant materials, a carbon of a little higher efficiency than that from rice hulls. Ceratophyllum and Opuntia dulcis are somewhat puzzling, but in the former we have low infusible ash in the plant, and high ash in the finished carbon, which probably accounts for its low efficiency in spite of the high nitrogen in the plant. In Opuntia dulcis, on the other hand, we have high infusible ash in the plant, but low nitrogen, and also relatively high ash in the carbon. The unknown Mexican plant, with only 15.26% ash, which, however, is largely infusible, gives a better carbon than might be expected, but here the ash in the carbon is quite low. The few remaining plants are low in ash and give poor carbons. Here again high nitrogen makes up for low infusible ash, as may be seen by comparing Pteris with Licania pyrifolia, for instance.

Taking it all in all, the agreement with former results is fairly good, especially when we consider that in plants we have to do with a mixture of different impregnating substances which may have low melting points, even though the components by themselves may not. And, furthermore, their effects need not necessarily be additive, but may in certain cases even be antagonistic.

Two of the plant materials mentioned in the above table were thought to form promising materials for carbons that might be produced on a commercial scale.

The decolorizing power of ordinary rice hull char was discovered by Blardone.[8] But the importance of rice hulls as a source of an efficient commercial decolorizing carbon was first recognized by Mr. W. G. Taggart,[9] Assistant Director of this station, and Dr. Schneller, Research Chemist at the time, made a detailed study of this problem. Rice hulls are obtained in immense quantities in the rice milling process. They find limited application as bedding for domestic animals, as packing material, as a soil ameliorant, and are used in large quantities as fuel in the rice mills. On account of the high silica content of the hulls, averaging 18.53%, while the total ash amounts to 19.54, the hulls are not entirely ashed in the process of burning, and the residue obtained usually contains from 12% to 25% of carbon. This material itself has only a very slight decolorizing power, but the latter can be greatly increased by removing the silica with an appropriate solvent, like caustic soda. When this is employed, silicate of soda is obtained as a by-product which will pay for part of the manufacturing expense. Instead of starting from the char obtained in the mills, the hulls may first be carbonized at a low temperature, and then retorted, just as is done in laboratory practice. The retorted hulls contain about 50% of carbon, and therefore give a better yield in finished decolorizing carbon than the char from the furnaces of the rice mills. Schneller made a series of experiments to find out how much caustic soda would be needed to prepare a commercial carbon of high decolorizing power. The results are shown in the following table:

TABLE XII.

Per cent NaOH	0	2½	5	10	15	20	30
Per cent ash in carbon	49.3	43.0	37.1	26.3	18.0	9.0	7.0
Per cent ash dissolved		6.3	12.2	23.0	31.3	40.3	42.3
Decolorizing power in per cent of Norit		17	25	67	90	145	145

The greatest decolorizing efficiency was obtained by boiling with 20% caustic soda, on the weight of the raw carbon. An increase of the amount of NaOH beyond 20% did not further increase the decolorizing power, and only slightly decreased the ash. This is probably due to the well-known fact that the solubility of silica decreases with the temperature to which it has been heated. Part of the silica in retorted char is heated higher than other parts, and this accounts for the difficulty experienced in removing the last few per cent of it.

Mr. Taggart has been awarded priority by the U. S. Patent Office for the decolorizing carbon prepared from rice hulls by the process just described. This patent when issued will be dedicated to the public.

The silica can also be removed practically completely by means of hydrofluoric acid. Two other methods, widely used in the manufacture of water glass, likewise give good results. In one experiment 10 parts of carbonized, but not retorted, rice hulls were mixed with 3 parts dry sodium carbonate, and the mixture heated in the muffle for two hours. After cooling, the mass was boiled out with water, washed with dilute hydrochloric acid and again with water. The efficiency of the carbon was 83%, and the ash content was 20.48. Still better results were obtained with the use of 5 parts of dry sodium sulfate instead of the sodium carbonate. The carbon made in this way had 7.87% ash, and an efficiency of 200. A carbon of even greater efficiency, 345, with only 3.50% ash, was prepared by heating the carbonized hulls with an equal weight of dry sodium sulfate, boiling out with water, then with caustic soda solution, washing with water, acid and water. It is doubtful whether any of these improved processes could find commercial application.

The second of the carbons which seemed to have commercial possibilities was obtained from Pacific Coast kelp. At the request of the senior author, Dr. J. W. Turrentine, in charge of the U. S. Experimental Kelp Potash Plant, at Summerland, California, sent us a sample of dried kelp.

In the first test this kelp, after thorough drying and grinding, was carbonized in an iron retort provided with an outlet for gases, until no more fumes were given off. The char was then transferred to a closed iron receptacle and heated for two hours to a bright red heat. It was then cooled, boiled out with hydrochloric acid, washed with water and dried. Upon examination it was found that the resulting carbon reduced the color of a molasses test solution to about one-third of that obtained by using an equal quantity of our standard carbon, Norit. A sample of kelp char, also received from Dr. Turrentine, when treated in a similar manner as the dried kelp, produced only a very poor carbon. We therefore decided to investigate this matter more thoroughly, and at our request Dr. Turrentine very kindly furnished us an ample supply of raw material for our further experiments, and we wish to express to him our thanks for this courtesy, as well as for the great interest he has taken in the progress of our work. The material received consisted of three different samples. The first, A, was kelp *(Macrocystis pyrifera)* dried in a rotary kiln; the second, B, was "incinerated" kelp, prepared as described below; and the third, C, was a sample made by subjecting kelp to destructive distillation. The last sample was kindly sent to us through Dr. G. C. Spencer, who was investigating the destructive distillation of kelp at the Forest Products Laboratory, Madison, Wisconsin. None of the three samples had been leached with water.

Upon investigation it was found that there are three factors which have an important effect on the properties of the final product sought. First of these is the particular point in the process at which the soluble salts and other ash constituents are removed. The second is the manner by which the material is carbonized. We used two different methods with Sample A. In one experiment it was charred in an open iron saucepan over a gas ring burner until fumes ceased to come off and the material was thoroughly carbonized. In other tests the process was conducted in a half gallon, heavy iron retort with a descending iron condensing tube about ½ in. in diameter and 3 ft. long. This was heated over a gas ring burner. Sample B, which was a char, had been prepared by feeding dried kelp into a revolving "incinerator," setting it on fire, and after it had been heated sufficiently, cooling it rapidly by quenching. Sample C was only partly carbonized, having undergone destructive distillation at a temperature not exceeding 314°C.

The third factor is the temperature to which the char, obtained by carbonization, is heated in a closed receptacle. We effected this final heating in the iron cylinder described before, made from a nipple of 2-in. pipe, and closed at both ends by screwed-on iron caps. This cylinder was placed in our muffle furnace.

Effect of Leaching. A part of each sample, A, B, and C, was boiled out several times with water, thoroughly drained, and again dried. Parallel experiments were then made with both leached and unleached material. The following table gives the tests and their results. The color determinations were here made by ordinary colorimetric measurements.

TABLE XIII.

TREATMENT	Efficiency of Carbon from Material Leached Before Heating	Efficiency of Carbon from Material Not Leached Before Heating
A, charred in retort, heated to bright red heat in closed iron cylinder, boiled out with water................	20	35
A, charred, heated to bright red heat, boiled out with acid, then water................................	36	80
B, heated to bright red heat, boiled out with water......	39	73
B, heated to bright red heat, boiled out with acid, then water..	68	294
C, heated to bright red heat, boiled out with water......	28	30
C, heated to bright red heat, boiled out with acid, then water..	54	73

The table shows that the better carbon is always obtained from the unleached material, and, in fact, the only carbon that is better than Norit, and considerably so, was prepared from material that was not treated with any solvent until after it had been brought to red heat. We may conclude from this that if our object is to make an active carbon, none of the mineral matter must be removed before heating the material to red heat.

Effect of Method of Carbonization. The way in which the kelp is carbonized is almost of as great importance as the question of leaching. The different methods of charring have already been described above. It is very difficult to carbonize the kelp in the iron retort always under the same conditions on account of varying gas pressure and because the condensing tube often gets more or less clogged with tarry products, thus preventing the free escape of the fumes. The effect of these factors, which were not under control, is strikingly shown in the figures below.

Table XIV.

Treatment	Efficiency
A, carbonized in an open saucepan, then heated to bright red heat in closed cylinder, boiled out with acid, then water	357
A, carbonized in iron retort, then heated to bright red heat, boiled out with acid, then water	322
Same, other experiment	200
Same, other experiment	133
Same, other experiment	80
B, heated to bright red heat, boiled out with acid, then water	294
C, heated to bright red heat, boiled out with acid, then water	73
C, first completely carbonized in open saucepan, heated to bright red heat, boiled out with acid, then water	59

These experiments show that the best results are obtained when the raw material is carbonized quickly at a comparatively high temperature and in such a way that the fumes can freely escape. Carbonization alone, however, is not sufficient to make an active decolorizing carbon, as is shown by the fact that Sample B itself, without first being heated to red heat, showed an efficiency of 22 when extracted with water, and of 64 when extracted with acid and then washed with water. Sample C gave 26 and 58 respectively.

Effect of Temperature to which the Material is heated after Carbonization. Three series of experiments were made to test this question, two (1 to 4 and 5 to 8) with char obtained by carbonizing Sample A in the iron retort at low temperature, and another with Sample B as received (9 to 11).

Table XV.

No.	Treatment	Efficiency
1	A, carbonized in iron retort, heated to full red heat, boiled out with water	35
2	A, carbonized, heated to medium red heat, boiled out with water	31
3	A, carbonized, heated to low red heat, boiled out with water	28
4	A, carbonized, heated to barely red heat, boiled out with water	24
5	A, carbonized, heated to full red heat, boiled out with acid, then water	80
6	A, carbonized, heated to medium red heat, boiled out with acid, then water	66
7	A, carbonized, heated to low red heat, boiled out with acid, then water	68
8	A, carbonized, heated to barely red heat, boiled out with acid, then water	58
9	B, heated to full red heat, boiled out with acid, then water, 294 and 333 average	313
10	B, heated to medium red heat, boiled out with acid, then water	160
11	B, heated to low red heat, boiled out with acid, then water	70

We find that within the temperatures at which tests were made the best carbon is obtained by heating to the highest temperature, full red heat. It is possible and even probable that still better carbons might be prepared by heating to even higher temperatures. One experiment was made in which a quantity of B was heated in a clay crucible in a Fletcher furnace, but observation showed that the temperature was not any higher than we could obtain with the iron cylinder in the muffle furnace. The resulting carbon, after washing with acid and water, had an efficiency of 280, which is very close to the 312 shown in the above table for the muffle heated carbon.

Another experiment was carried out in order to see whether a good carbon could not be made in one operation. The iron cylinder described above was filled with dry kelp, and one of the caps was only screwed on loosely, so that the fumes might escape, without giving the air free access to the char. After heating to full red heat the carbon was boiled out with acid, and washed with water. It had an efficiency of 133, and was therefore much less effective than the carbon produced in two operations.

We have also found that it is not necessary to extract the carbon directly with hydrochloric acid. The water-soluble salts can first be removed with this solvent, and the greater part of the remaining ash is then dissolved with hydrochloric acid, after which the acid is again washed out with water.

Summarizing briefly, our tests have shown that a carbon which has a much greater decolorizing power than Norit can be prepared in the laboratory by quickly carbonizing dried Pacific Coast kelp in such a way that the fumes can freely escape. After they cease to come off, the char is transferred to a closed iron receptacle and heated for 2 hours or so to red heat. Instead of charring dried kelp, "incinerated" kelp may be used directly. The carbon is then boiled out either with dilute hydrochloric acid or first with water and then acid. This is again washed out with water, and the carbon dried. It remains to be seen whether the process can be worked successfully and economically on a large scale, and whether the price to be gotten for the finished product will warrant its manufacture.

The U. S. Experimental Kelp Potash Plant has kindly taken up this work on a semi-commercial scale, and it is very gratifying to report that satisfactory progress is being made in these tests. U. S. Patent No. 1,290,002, covering the kelp carbon, has been recently granted to the senior author, who has dedicated it to the public.

B. CARBONS FROM BY-PRODUCTS AND SIMILAR MATERIALS.

The by-products of the cane sugar industry to be considered in this connection are bagasse, filter press cake, and molasses. The use of bagasse char was first proposed by Weinrich in 1891.[10] Purification of this char by means of caustic soda was advocated by Clacher in 1914.[11] Bagasse has a low ash content, only about 5%. It is therefore not surprising that the carbon made from it, after treatment with caustic soda and then hydrochloric acid, showed an efficiency of only 26, its ash content being 6.33%. It should be remarked, however, that bagasse might be used just as well as sawdust to form the raw material in some impregnation process; for instance, with lime.

Carbon from beet press mud was first recommended by Karlik and Stanek[12] in 1903, and that from cane press mud by Hazewinkel[13] in 1911. Sandmann[14] went a step further and purified his carbon by means of hydrochloric acid. For details about Sandmann's process, Bulletin 161 of this station may be consulted (pages 7 and 13).

Filter press cake contains a large quantity of organic matter, and is naturally impregnated with varying amounts of lime. The decolorizing power of the carbon made from it depends largely on the ratio of lime salts to organic matter, on the nature of the lime salts, and on the ash content of the finished carbon. At this station we have prepared carbons from seven different samples of press cake, treating the carbon only with hydrochloric acid. One of these samples was from Chaparra, Cuba, and represented ordinary defecation cake. The efficiency of the carbon made from it was 105, and the ash content 21.62%. Cake from the sulfitation process, obtained at our sugar house in 1917, gave a carbon of an efficiency of 173, and with 52.27% ash. Press cake prepared in our experimental runs of the 1918-19 grinding season (see La. Bulletin No. 165) yielded the following results: Carbon from run 1, efficiency 109, ash 73.47%; from run 2, efficiency 69, ash 74.22%; from run 3, efficiency 118, ash 66.29%;

from run 4, efficiency 146, ash 64.78%; from run 5, efficiency 50, ash 76.69%. It would appear that carbon from press cake high in phosphate of lime (run 4) is better than that from cake low in lime phosphate (runs 1 and 3). This confirms previous results. It is also shown again that better carbons are obtained when the ratio of impregnating material to organic substance is high (runs 1, 3, and 4) than where it is low (runs 2 and 5). Filter press cake contains considerable quantities of silica. This explains the high ash content of the carbons purified only with hydrochloric acid. Carbons with less ash and of higher efficiency could be prepared by purifying the raw carbon first with caustic soda.

Final cane molasses is low in ash, from the standpoint of carbon manufacture. It must therefore be impregnated in order to produce a good carbon. Lyon and Peck (U. S. Patent No. 1,251,546) advocate the use of concentrated sulfuric acid as impregnating material, and for certain purposes, the additional use of kieselguhr and similar materials. In experiments made in this laboratory a sample of final molasses, carbonized simply by mixing with an equal weight of concentrated sulfuric acid, gave a carbon which, after washing with water, showed a decolorizing power of 42, and an ash content of 0.52%. A better carbon was obtained by assisting the carbonization with gentle heating. The carbon produced in this way had an efficiency of 52, and an ash content of 1.50%. Peck and Adams have furnished a very interesting report on decolorizing experiments with this molasses carbon.[15] It appears very promising, especially on account of its cheapness.

Weinrich (private communication) uses lime as impregnating substance, and where a hard, granular substitute for bone-black is desired, further additions of insoluble material like clay, kieselguhr, etc., are employed. When a bone-black substitute is made, the mineral skeleton is only partially or not at all removed, but when decolorizing carbon is to be prepared, the impregnating substance is dissolved with water and hydrochloric acid. Mr. Weinrich has kindly furnished us a detailed description of his process, which is to be patented, and also supplied us with samples of all the products made by him. All of these samples were found greatly superior to bone-black in decolorizing power.

Considering them from the standpoint of what is expected of decolorizing carbons, the samples which retain the mineral skeleton are poor. The first three samples of this class showed an efficiency of 54, 30, and 25 respectively; another sample received later gave 26. All of these carbons contained lime in such a form that they would render our products alkaline, which is, of course, undesirable. But recently we received a sample of acid-proof char, with mineral skeleton, recommended as a substitute for bone-black and therefore in granular form. This showed an efficiency of 34. At the same time, Mr. Weinrich also sent us a sample of decolorizing carbon made by his process. This had an efficiency of 59, and was therefore superior to Lyon and Peck's molasses carbon. It would appear, however, that it will be more expensive to produce.

Some carbons have also been made at this station from a raw material and an impregnating substance which are found side by side in nature. In some districts in Porto Rico there exist certain marshy areas, in which the upper stratum, varying in thickness from a few inches to a number of feet, consists of a black vegetable mould. Directly below this is a white to yellowish material which is almost pure carbonate of lime, in a very fine state of division. The average composition of the black layer is, on a moisture-free basis, 27.4% ash, 14.0% infusible basic oxides, 9.69% silica, and 1.43% nitrogen. The figures for the light material are 54.8,% 53.5%, 0.3%, and 1.0% respectively. It would be expected that good carbons should be obtained from mixtures of the dried materials in proper proportions by subjecting the latter to our regular process, and purifying the raw carbon with hydrochloric acid. In fact, two parts of light material and one of black furnished a carbon of 181 decolorizing power, and 40.10% ash. The carbon made from equal parts of the two ingredients showed an efficiency of 106, and an ash content of 32.79%. When using one part of light material to two of black, the efficiency of the carbon was still lower, 67, and the ash amounted to 30.15%. These results again show the effect of the proportion between impregnating substance and organic material. The high nitrogen content of the vegetable mould is probably also partly responsible for the high decolorizing power of the carbon.

CONCLUSION.

The conclusions arrived at from the results of our impregnation tests on sawdust and similar materials have been summarized on pages 28 and 29. Our additional experiments with materials which contain at the same time both the necessary organic matter and the impregnating substance have further strengthened our deductions. We have attained our three principal objects—first, to prepare a carbon of exceptionally high decolorizing power for research purposes; second, to detect relations between method of preparation and the decolorizing power of the carbons; and, third, to find cheap and abundant raw materials for the commercial manufacture of decolorizing carbons.

We have already mentioned that a knowledge of some hitherto undetermined physical constants, and further chemical data are needed for a complete interpretation of our results. Particularly, additional information should be sought concerning the effect of mixtures of various impregnating substaces. Tests along these lines, and investigations of other minor factors, would probably furnish valuable information by which it would be possible to explain the remaining slight discrepancies found in the decolorizing power of the carbons made from some plant materials. As far as our investigations are concerned, this work would lead us too far astray from our principal objects, and we shall therefore have to leave this field to others who may be more especially interested in this phase of the subject. Our further researches can be based on the results already obtained, and will deal with the fundamental principles underlying decolorization; that is to say, with the properties of the carbons themselves which are related to and responsible for their effect. We confidently hope that the knowledge to be gained by these researches will prove of benefit to the industry for which we are laboring.

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Agricultural Experiment Station

OF THE

Louisiana State University and
A. & M. College,

BATON ROUGE

ANTHRAX

TRANSMISSION OF INFECTION
BY
NON-BITING FLIES

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Anthrax: Transmission of Infection by Non-Biting Flies.

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During 1918 the Department of Animal Pathology of the Louisiana Experiment Station published bulletin No. 163 entitled, "Blood-sucking Insects as Transmitters of Anthrax". This publication was a report of a series of experiments in which certain blood-sucking flies and mosquitoes were proven to be transmitters of anthrax.

Since the issuing of that report a great many inquiries have been rceived by the department concerning the non-biting insects as possible carriers of anthrax from infected carcasses to living animals; also the possibility of blow-flies carrying the disease after emerging from carcasses of animals dead of anthrax.

In a review of the literature on anthrax very little could be found concerning the direct transmission of anthrax by non-biting insects. A great many workers have recovered anthrax bacilli from the bodies of flies after having fed upon anthrax infected blood. Anthrax bacilli have been recovered from blow flies which emerged from larvæ fed on meat infected with anthrax spores.

These results have all been obtained by foreign workers. There seems to be no report of any work carried on under conditions such as are found in the anthrax areas of America.*

Enzootics of anthrax in Louisiana usually occur during the summer months while all species of flies are prevalent. Frequently the carcasses of animals dead of anthrax are not properly destroyed and maggot flies develop in great numbers. Sometimes, through ignorance, the skins are removed. It has long been the practice in some sections of the state to scarify anthrax swellings on horses and mules allowing the blood to escape. This is a very dangerous practice as the escaping blood and serous exudate are filled with anthrax bacilli.

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*(Pierce. "Diease Borne by Non-biting Flies" Lecture 8 Proceedings of the Class Formed to Study the Entomology of Disease, Hygiene and Sanitation in manuscript 1918).

With these sources of infection present during outbreaks of anthrax, it was thought possible that the non-biting insects might be transmitters of the disease. A study of some of the common insects as carriers of anthrax was undertaken in the pathology laboratory of the Louisiana Experiment Station and the following is a report of the work.

The purpose of the experiments was to test the possibility of flies, after having fed upon anthrax-infected material, carrying infection to wounds on healthy animals, also the possibility of flesh or maggot flies carrying infection after being developed in anthrax carcasses.

The flies used in these experiments were the house fly, *Musca domestica* and the maggot flies, commonly called "blue bottle" and "green bottle" flies, *Calliphora erythrocephala, Lucilia caesar, Lucilia sericata.**

TECHNIQUE

Guinea pigs were inoculated with a loop of anthrax spores injected subcutaneously or into the abdominal cavity. These inoculated guinea pigs were used as sources of infection. In some cases a piece of liver or spleen from a carcass was used as infected material while in others the external swelling on the living pig. A microscopic examination of all material was made to determine the presence of anthrax bacilli. The flies were bred in breeding cages. A single fly was placed in one of the large wide-mouthed sterile test tubes which were used as retainers for the flies. A guinea pig was secured on a small table with the abdominal surface up and the hair removed. An incision about an eighth of an inch long was then made through the skin.

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*These determinations were made by Mr. F. C. Bishop, U. S. Bureau of Entomology). All experiments were conducted with guinea pigs as experimental animals under as nearly natural conditions as possible.

A very small piece of anthrax-infected liver or spleen was placed in a sterile petri dish. Over this piece of anthrax material a test tube containing one fly was inverted. The fly was observed closely until it descended into the dish and either fed or walked over the anthrax infected material. In some experiments the fly was transferred directly to the the susceptible animal and observed until it visited the skin wound. In others a period of time was allowed to elapse between the feeding on the infected material and the susceptible animal. In the latter experiments the fly was transferred to another sterile test tube before feeding upon the susceptible animal. This was done to eliminate the possibility of carrying anthrax infection by a contaminated test tube.

After death post mortems were made of all guinea pigs and bacteriological examinations made of the blood taken from the spleen. All doubtful cases were cultured and animal inoculations made to prove results.

The results of these experiments are given in Table I.

TABLE 1.

ANTHRAX TRANSMISSION BY NON-BITING FLIES.

Number of Pigs	Insect Used	Time between feeding and contact with wound	Number dying with Anthrax	Percentage of Infection
32	House fly	0	15	46
15	Blue bottle fly	0	6	40
12	House fly	5 minutes	9	75
12	House fly	20 minutes	6	50
6	House fly	80 minutes	3	50
8	House fly	240 minutes	2	25
8	House fly	300 minutes	2	25

Table No. 1, shows that a single fly feeding upon flesh from an anthrax carcass transmitted the disease to a guinea pig through a small skin lesion. The results show that a fly could transmit the disease for a period of five hours after having fed upon infected flesh.

In most cases the external or carbuncular form of anthrax was produced. The guinea pig died anywhere from forty-eight to seventy-two hours after being exposed to the infected fly.

The transmission of the disease was mechanical, the anthrax bacilli being carried from the infected material to the susceptible animal on the soiled proboscis and feet of the fly. In some cases infection would be produced by the fly walking over the wound on the susceptible animal.

LIVING ANIMAL AS A SOURCE OF INFECTION

In another experiment a live guinea pig sick with the external form of anthrax was used as the source of infection. Method:- Several guinea pigs, including the infected one, were secured on small tables with the abdominal surfaces up. A small incision was made through the skin into the carbuncular swelling on the infected animal. Through this incision a drop of serous fluid would escape which was used as the source of infection. The same technique was used as in the preceding experiment. The house fly and stable fly (*Stomoxys calcitrans*) † were used in this experiment. The results are given in Table 2.

TABLE 2.

ANTHRAX TRANSMISSION BY FLY FROM GUINEA PIG

Number of Guinea Pigs	Insect Used	Time beween feeding and contact with wound	Number dying with anthrax	Perectage of Infection
18	House fly	0	7	39
6	Stable fly	0	1	16

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†While **Stomoxys calcitrans** is a biting fly, it was used as a mechanical carrier in the same manner as the house fly (**Musca domestica.**)

Table 2. shows that a fly after feeding on the serous discharge from an opened anthrax swelling transmitted the disease to a healthy guinea pig by feeding upon a skin wound.

The opening of anthrax swellings on animals is a very dangerous and useless practice. It not only produces a source of infection for insects but every drop of the bloody discharge contains anthrax bacilli which contaminates the grass and soil wherever dropped.

ANTHRAX IN DECOMPOSING CARCASSES

In certain parts of Louisiana, especially in the forest and swampy areas, carcasses of animals which die of anthrax are frequently overlooked and are left undestroyed until destroyed by carrion eating animals, birds and insects. The possibility of the spread of anthrax by carrion feeders has been reported in Bulletin 136 of this Station. The possibility of insects breeding in these carcasses and then carrying the disease to other localities has never previously been carefully investigated in this region.

In order to determine the role of insects in the dissemination of anthrax from anthrax carcasses, a series of experiments were conducted during the years 1918 and 1919. Some interesting results were obtained in regard to the time the vegetative and spore stages of anthrax are able to survive in decomposing carcasses at summer temperature and also the relation of maggots to the spread of the disease. The flies used in these experiments were *Calliphora erythrocephala, Lucilia caesar* and *Lucilia sericata,* commonly known as blue bottle and green bottle flies.

Gunea pigs which had died of anthrax were used as breeding material for the flies. The experiments were carried on in breeding cages prepared especially for this work. The dimensions of the cages were 12x12x12 inches. The sides were of glass and the top consisted of a closely fitting sliding door. The ventilator was covered with a fine copper wire screen, forty meshes per inch. This cage gave complete protection against gnats which gave considerable trouble during the early work on this problem. Two inches of sand were placed in the bottom of each cage on which a guinea pig dead of anthrax was laid. Eggs or young larvæ of flies which had been deposited upon a carcass free of anthrax were placed upon the anthrax infected carcass in the cage. This method was followed to reduce the chances of spreading the disease while obtaining an infestation of maggots. Ten different tests were made with the maggot fly during the summer months. In each test three guinea pigs were inoculated with a suspension of anthrax spores. After death the carcasses were disposed of as follows: one was opened, a blood smear made and examined for anthrax bacilli. If the diagnosis was positive the other two carcasses were placed in breeding cages. On one was placed a number of fly eggs, while the other was kept free of maggots as a check. As soon as the maggots developed, a number were collected and cultures made to determine the presence or absence of anthrax bacilli in or on their bodies.

Method—First. A maggot was placed in a sterile plate and allowed to crawl over the surface of the agar culture media. Second. A maggot was placed in a test tube containing ten cubic centimeters of sterile water. Agar dilution plates were made from the contents of this tube in the usual manner.

Fifteen maggots were collected from each anthrax carcass and cultures made as described above. No colonies of anthrax developed in cultures.

The interior of the maggot was examined for anthrax bacilli in the following manner: A maggot was placed in a solution of bichloride of mercury and allowed to remain in the solution for ten minutes. It was then placed in a sterile dish, torn open with sterile forceps, and cultures made from the interior, as described above.

The same number of maggots were cultured as in the preceding experiment with the same result—no anthrax colonies developed.

At the time the maggots were examined the check carcasses were also opened and cultures made from the liver, spleen and heart. No anthrax developed in any of the plates.

The pupal stage of the fly would usually last from two to three weeks. As soon as the flies appeared in the cage, fifteen were collected and cultures made from their bodies to determine the presence or absence of anthrax becilli. Practically the same technique was used as with the maggots. One hundred and fifty flies were examined. Results were negative. No colonies of anthrax developed in the cultures.

The results of the above experiments would indicate that flies breeding in unopened carcasses of animals dead of anthrax during the summer months in Louisiana do not carry anthrax bacilli on or in their bodies. It would appear that the vegetative stage af anthrax is destroyed during the summer months in Louisiana in the unopened carcasses by the process of decomposition. Spores of anthrax do not form in the unopened carcasses.

It is probable that the same conditions would not be found present during the colder weather of the winter months. Decomposition is much slower at low temperatures. This was tested out in December and January. Ten carccasses of guinea pigs dead of anthrax were placed in a cool unheated room for a period of twenty days. At this time the carcasses had partly dried up. Cultures made from these pigs, from time to time, always showed the presence of anthrax.

Spores, however, develop very quickly on the surface of any opening in the carcass, not only in the natural openings, but also in cuts and injuries of various kinds. Carcasses are frequently torn open by carrion feeders, thus allowing abundant spore formation. On account of the resistance of the spores of anthrax, it was necessary to run some experiments with the flies on carcasses that had been opened and in which spores had developed. A few experiments were conducted along this line.

Anthrax carcasses were opened by cutting away the abdominal wall. The carcasses were infested with the eggs or young larvæ of the flies. The same method was followed as in the preceding experiment. Fifteen maggots and the same number of flies were collected from each carcass. Five carcasses were used in the experiment. Results: Cultures of anthrax were recovered from ten maggots. No anthrax was recovered from the flies.

EFFECT OF DECOMPOSITION ON ANTHRAX SPORES

A few tests were conducted to determine the effect of animal decomposition upon anthrax spores. Method: A suspension of anthrax spores was injected into the carcass of a healthy guinea pig. The carcass was placed in an incubator at 35°C and allowed to remain for twenty days. It was then opened and cultures made from the contents of the abdominal cavity. Anthrax colonies developed in every culture.

Five carcasses were prepared in the same manner as above, placed in the breeding cages and infested with eggs or young larvæ of the bluebottle fly. Twelve maggots, and the same number of flies, from each cage were cultured as in the previous experiments with the following results: Anthrax was recovered from the outside and inside of every maggot examined. Anthrax was recovered in like manner from all the flies examined.

Three pupæ were placed in a large test tube partly filled with sterile sand. After the flies had hatched, the puparia were collected and cultured. Colonies of anthrax developed in every culture.

The results of the above experiments would indicate that the spore form of anthrax is not destroyed by the process of decomposition of the animal carcass. Also that flies bred in the presence of anthrax spores do carry infection on and in their bodies.

SUMMARY AND CONCLUSIONS

Briefly, the results of the experiments described above may be summed up as follows: 1. The house fly, *Musca domestica,* and the "blue bottle", or "green bottle" flies, *Calliphora erythrocephala, Lucilia caesar* and *Lucilia sericata* are capable of carrying anthrax infection to wounds on healthy animals, after having fed upon anthrax-infected flesh. 2. The House fly and the stable fly, *Stomoxys calcitrans,* are capable of carrying anthrax infection to wounds on healthy animals after having fed upon the discharges from an open carbuncular swelling on an animal sick with anthrax. 3. Flies bred out of an unopened anthrax carcass during the summer months in Louisiana do not carry anthrax infection. 4. The results would indicate that flies bred in the presence of anthrax spores do carry anthrax infection. 5. The results would indicate further that the vegetative form of anthrax in the unopened carcass is destroyed by the process of decomposition.

In this report on the subject of anthrax transmission by non-biting flies, the results obtained apply to conditions as found in Louisiana.. The results of this work should prove the necessity for prompt and complete destruction of all anthrax carcasses. They should also help to educate the people against the dangerous and useless practice of opening anthrax swellings on animals sick with the disease. The results should prove the necessity of giving animals greater protection against the ravages of all insects during outbreaks of anthrax.

ACKNOWLEDGEMENTS

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Baton Rouge

Effect of Fungi on the Germination of Sugar Cane

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Baton Rouge
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EFFECT OF FUNGI ON THE GERMINATION OF SUGAR CANE.

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One of the greatest losses to the sugar industry of Louisiana is that caused by the poor germination of the seed cane. This is much more important to the planters in this state than it is in tropical sugar countries. In the tropics the planters are usually able to cut their cane in pieces and then plant the pieces but slightly thicker than they want the hills of cane. In contrast to this, the Louisiana planter must put down at least two solid rows of stalks in each furrow to obtain a satisfactory stand. To do this it takes about four to five tons of cane per acre for planting. Or in other words, if the same acreage is maintained, it takes nearly one-sixth of the crop each year for planting purposes.

In the tropics, the young plants come up quickly after the seed is planted, with the result that a comparatively high percentage of germination is obtained. There is some deterioration of seed, especially in those regions in which the soil is badly contaminated with the pineapple disease fungus, *Thielaviopsis paradoxa,* yet the deterioration averages very much less than it does in the southern United States. According to various statistics (16), the percentage of germination of the eyes or buds in the tropical countries usually runs from 45 to 90%.

Under Louisiana conditions, the cane is planted in the fall or kept in windrow until early spring and there is no chance for satisfactory germination until the warm weather begins in the spring. Instead of a few days or weeks as in the tropics, the cane in Louisiana must lie in the ground for several months. During this period there is continuous deterioration due to the action of various micro-organisms aided to a greater or less extent by unfavorable soil and temperature conditions. As a result, instead of a comparatively high germination of the eyes as is obtained in the tropics, we actually obtain about 10 to 30% germination with an average of about 20%. This means that we have to plant about five times as much cane as would be necessary if we were able to obtain a complete germination of the eyes.

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*The authors wish to acknowledge their indebtedness to Mr. W. G. Taggart, Assistant Director of the Sugar Experiment Station at New Orleans, who has helped in all the work at Audubon Park and has materially assisted in obtaining the data given in this bulletin.

In the tropics, also, the planter is able to use cane tops to a considerable extent for planting. The tops seem to germinate better and are less liable to be affected by the various diseases. This, however, cannot be done satisfactorily in Louisiana. On account of the large tonnage that must be handled, the extra labor would be prohibitive. Furthermore, under the conditions existing here, deterioration is more pronounced with the tops than it is with the more mature parts of the stalks. That this is true is shown by the experiments which have been carried on at New Orleans during the past few years. Stalks have been cut in two and the bottom halves and top halves have been planted in separate rows. All the good eyes were counted at planting time and the percentage of germination obtained the following spring. The results of the tests are given in table 1. The figures represent the percentage of sound eyes that grew into plants.

TABLE 1.

Germination percentage of top and bottom halves of stalks.

YEAR	D 74		PURPLE	
	Bottom Halves	Top Halves	Bottom Halves	Top Halves
1911	10.0%	22.0%	10.0%	18.0%
1912	36.0	21.0	33.0	28.3
1913	23.1	23.1	21.8	10.4
1914	25.2	17.7	9.9	12.7
1915	18.7	11.1	22.3	16.3
1917	12.2	6.5	19.2	21.0
Average	20.9	16.9	19.4	17.8

While the tests show a considerable variation from year to year, the average for the six years shows a greater germination of the eyes on the bottom and more mature halves of the stalks.

The problem of the deterioration of seed sugar cane is an extremely complex one. While the actual rot or decay of the cane is due to the action of various species of fungi and bacteria, there are many factors which influence the rate and extent of the deterioration. Among these should be mentioned the nature of the soil, moisture conditions of the soil at various times during the winter, temperature conditions, depth at which the cane is planted, maturity of cane at time of planting and the condition of cane in regard to infestation with insects and fungi. With our present knowledge of the problem, it is impossible to estimate the actual importance of any one of these various factors.

The problem of seed deterioration has received considerable attention at different times in the various tropical sugar countries. The various investigators have considered the problem mostly from the standpoint of the organisms that were found developing on the cane and the possibility of treating the cane in order to prevent the entrance of the organisms. In fact, this seems to be the best way of approaching the problem. It is necessary at first to have a knowledge of these organisms and their methods of attack before going deeper into the problem. Investigators who have made a study of the organisms causing seed deterioration include Went and Kamerling in Java, Cobb and Lewton-Brain in Hawaii, Bovell, Howard, Watts and Johnston in the West Indies and Butler in India. The organisms which received particular attention by them were the ones causing the pineapple disease, red rot, rind disease and the root rots. Reference will be made to their work from time to time throughout this article.

The problem of the deterioration of seed sugar cane has been under investigation at the Louisiana Experiment Station for a number of years. The work has included principally a study of the various organisms found on seed cane, the loss caused by them, and their methods of attacking the cane, although various other factors have been kept in mind as far as possible. The experimental work has been carried on in the field and laboratory of the Louisiana Experiment Station at Baton Rouge and in the field at the Sugar Experiment Station, Audubon Park, New Orleans.

METHODS OF WORK.

The work has been carried on along three general lines. (1) *Examination of planted cane for the presence of disease organisms.* During the course of the investigation, a large amount of seed cane, especially cane which has failed to germinate, has been carefully examined and the different fungi present have been listed and their effect on the cane has been studied. Most of this cane has come from Louisiana but some has come from other southern states. (2) *Inoculation experiments.* Most of the fungi which seemed to be important have been isolated and inoculation experiments have been carried on in cane at planting time. Also some of them have been used to inoculate growing cane in order to obtain seed that had been infected for some time. The effect of these different organisms on germination of the cane has been determined. (3) *Effect of disinfectants on seed cane.* Cane has been treated at planting time with different disinfectants such as corrosive sublimate and formaldehyde to see if these would have any effect on the germination.

Fungi on Seed Cane.

Cane that has lain in the ground all winter is always more or less infected with various fungi and bacteria. The cane that does not germinate is always badly discolored on the inside, and the outside of the stalks is frequently shrunken and shows the presence of fungus fruiting pustules. The color of the inside of the stalks is not constant but is usually brownish or reddish, though blackish, yellowish or greyish discolorations are not uncommon. The canes in Plate I show a common appearance of deteriorated cane during the late winter and early spring seasons.

The fungi which have been found on such cane and which from their frequency or from their known ability to infect cane seem important include the following: *Colletotrichum falcatum, Melanconium sacchari, Gnomonia iliau, Marasmius plicatus, Thielaviopsis paradoxa, Fusarium sp.* (two or more), and *Scopularia sp.* A large number of other forms have been seen on cane or have been isolated while making cultures from stalks. These include *Sclerotium rolfsii, Pythium artotragus,* and species of the following genera, *Agaricus, Rhinotrichum, Alternaria, Penicillium, Aspergillus, Meliola, Rhizopus, Cladosporium, Mucor, Saccharomyces, Acremonium, Catenularia, Nectria* and others. While it is possible that some of the latter may have some importance in the deterioration of cane, it does not seem likely and consequently not much attention has been paid to them. They seemed to be mostly saprophytes growing on the already dead tissues of the cane. Bacteria were also present in abundance in most of the canes examined. While it is quite possible that some of these may aid in the deterioration of the stalks and eyes, they did not seem to be particularly important and were not considered to any extent in this investigation.

COLLETOTRICHUM FALCATUM

Colletotrichum falcatum is the cause of the well-known red rot disease of sugar cane. This fungus causes a disease of the growing cane but it is also very abundant on seed cane and is one of the chief organisms responsible for seed deterioration. This fungus was first reported in the United States in 1911 and has since been found to be well distributed over the sugar sections of this country. It is probable that it has been here for a considerable period. This fungus seems to be present in all tropical sugar countries and in many places it does considerable damage. The red rot disease, as it occurs in Louisiana on growing cane and to some extent on the seed, has been discussed in previous publications (8, 9, 11). The fungus usually gains entrance to the living cane through borer holes or other injuries. The rind tissue is not usually attacked unless it is very young and tender. As the disease is usually confined to the interior of the stalk, it is best told by examining a stalk that has been split lengthwise. The inside of the stalk is much reddened and in this red tissue are elongated white areas at right angles to the main axis of the stalk. The presence of these peculiar white spots is conclusive proof of the presence of the red rot disease (Plate IV, fig. 1; Plate V, figs. 1 and 2).

In seed cane, the presence of the fungus is not always readily told by a microscopic examination. The white spots may be present, but their absence does not mean that the red rot is not present. The action of *Colletotrichum falcatum* in cut stalks is somewhat different than in growing stalks. Furthermore, as this fungus becomes combined with other organisms in the deteriorating cane, the interior of the stalks may assume various colors. The tissues may become reddish, brownish or greyish. The nodal region of a stalk of seed cane affected with this fungus is usually brownish in color and it is from this region that cultures of the red rot are more liable to be obtained, especially if the stalk has been infected for some time. The buds on these infected nodes are usually dead. Figure 2 in Plate III shows split stalks that have lain in moist chamber for a few days. The developing mycelium in the nodal region is that of *Colletotrichum falcatum*.

While the fibrovascular bundles are always reddened in standing stalks that are affected with the red rot, this is not always the case in seed cane. Very frequently the bundles are perfectly colorless or at most but slightly reddened.

The presence of this fungus shows but very little on the outside or rind of the affected seed stalks. While many seed stalks, especially immature ones, shrivel to some extent, this is generally due to the action of some other organism than the red rot fungus. About the only evidence of *Colletotrichum falcatum* on the rind is the presence of blackened rootlet buds in the nodal region. There are small thin places in the rind at each node where the young roots emerge. It is apparently through these points that *Colletotrichum falcatum* gains entrance to the seed cane. This method of infection was first demonstrated by Butler (3) in India. From a large number of observations in Louisiana, it seems evident that much of the infection takes place in this manner. Infection through the cut ends of the stalks does not seem important probably because these cut ends quickly become infected with yeasts and various other organisms that prevent the development of the red rot fungus.

There has been some discussion by different investigators regarding the possibility of young cane plants becoming infected with the red rot disease directly from diseased seed. Butler (2) in 1906 claimed that in India the mycelium of *Colletotrichum falcatum* passed directly from the diseased seed into the young plants and he claimed that most of the growing plants became infected in this manner. This question was investigated a few years later in Louisiana and in Bulletin 133 (9), this method of infection was denied for our conditions. While it was admitted in this bulletin that diseased seed cane showed a lower germination of the eyes, it was claimed that the plants that did develop did not become infected from the seed. Following this, the problem was considered in the West Indies (32) with results very similar to those obtained in Louisiana. After this, Butler (3) again worked over the whole problem in India. His results agreed with those he had given in his earlier publication. He showed definitely that the plants do become infected from the diseased seed.

From the various publications on this subject it is evident that for some reason or other, the conditions in India are radically different from those in the West Indies and the southern United States. In our experiments during the last few years in which thousands of diseased stalks have been planted, not a single stalk has been seen which became infected directly from the seed. Just why the fungus acts differently in the different countries is not known. It may be that there are different strains of the fungus in the various sugar countries or it may be due to varietal susceptibility or climatic conditions.

Colletotrichum falcatum occurs on a large part of the seed cane in Louisiana. It is one of the most widely distributed and common forms found on cane. It is usually easy to obtain cultures of the red rot from even a small sample of seed cane that has lain in the ground during part of the winter.

MELANCONIUM SACCHARI.

Melanconium sacchari is the cause of the so-called rind disease of sugar cane. This fungus is widely distributed in all sugar cane regions of the world. The early investigators (23) of sugar cane diseases believed that this fungus caused a very serious disease of standing cane. They believed that the deterioration and dying out of some varieties, especially the Bourbon, was mainly caused by this fungus. This belief has been largely discredited. It is the general opinion at present that most of the deterioration noted by these early workers was caused by the red rot and various root rots and that *Melanconium sacchari* was merely secondary. However, Johnston (18) still believes that the rind disease is of considerable importance in the West Indies.

In Louisiana it is very unusual to see this fungus on standing cane. Sometimes it may be seen on stalks that are practically dead from other troubles but even this is unusual. However, on the seed cane during the winter months it is very common and abundant. It is often difficult in the late winter to find a stalk that does not show the presence of the organism on some joint. The presence of the fungus is very readily told. The fruiting pustules break out through the rind tissue and are readily seen or can be felt by running the hand over the surface. Affected stalks are usually more or less shriveled due to the decay of the tissues. The inside of the stalk is not very characteristic. As the stalks lose moisture rapidly, a sort of dry rot develops and the tissues do not have the turgid, moist appearance that is present with the red rot. The decaying tissues become whitish, greyish or brownish. A red discoloration is not characteristic of this trouble though some red may be present.

MARASMIUS PLICATUS.

Marasmius plicatus is the cause of the root rot disease of sugar cane in Louisiana. This is not the same fungus that is responsible for most of the root rot troubles in the tropics, though it seems probable that this fungus is present more or less in the West Indies. In the southern United States this fungus has gone under the name of *Marasmius plicatus* since Fulton (13) made a study of it in Louisiana. In a recent article, however, Johnston (17) claims that it is identical with *Marasmius stenophyllus,* a fungus which is common in the West Indies on bananas and to some extent on sugar cane. It is possible that Johnston is right, yet it does not seem that the point is well enough settled to make it advisable to use the other name. It is a little strange that the fungus should be so abundant on sugar cane in Louisiana and to prefer another host in the West Indies. Whether *Marasmius plicatus* is the right name for the fungus is questionable but as it is the name that has been used for some time in Louisiana, it will be used in this bulletin.

Marasmius plicatus causes a disease of the growing cane (Plate IV, fig. 2), but as diseased stalks are liable to be used for planting, it is frequently seen on seed cane. It attacks the roots of the growing plants and also grows around the base of the stalks. It grows in and between the lower leaf sheaths of the stalk and the abundant mycelium cements these together so that they remain on the stalk all through the growing season. The presence of the disease is easily recognized in the field by pulling apart the leaf sheaths at the base of the stalks. If abundant mycelium is found between the leaf sheaths and the stalk itself is unaffected, root rot is present. The fungus does not penetrate the rind tissue from the leaf sheaths.

Stalks affected with the root rot are easily recognized in seed cane by the presence of the clinging leaf sheaths and white mycelium. The inside of the stalk is not affected, though mycelium is sometimes found entering the stalk in borer holes and other injuries.

The fungus forms the mushroom fruiting bodies in the field from July to September. It does not fruit on the seed cane.

GNOMONIA ILIAU.

Gnomonia iliau is the cause of the Iliau or stem rot disease of sugar cane. This disease is only known in Hawaii (25) and Louisiana (10). It was first described from Hawaii where it is commonly called Iliau, meaning hidebound.

The Iliau disease (Plate II, fig. 1) is principally a disease of the growing cane. In some respects it appears very similar to the root rot disease. The lower leaf sheaths are bound together but they are also bound firmly to the stalk itself. This fungus, unlike the root rot fungus, grows into the stalks from the leaf sheaths. As soon as the fungus begins to fruit, it can be readily told from the outside by the numerous black perithecia which develop. The beaks of the perithecia are long, hard and sharp pointed.

The fungus occurs mostly in the north and central parts of the state and is not common in the southern section, though it has been collected as far south as Reserve.

As diseased stalks may be cut at planting time, affected stalks are sometimes found in the seed. The presence of this fungus on the seed cane can be readily told. The lower leaf sheaths remain firmly attached to the stalks and, differing from the root rot, the rind tissue and also the interior of the stalks are affected.

THIELAVIOPSIS PARADOXA.

Thielaviopsis paradoxa is a common fungus in many tropical countries where it is the cause of the so-called pineapple disease of sugar cane. It received this name from the peculiar pineapple odor that is present in the affected stalks. In the tropics, this disease is apparently the cause of a large part of the deterioration of seed cane.

The fungus enters wounds in the rind tissue or at the cut ends and rapidly grows through the whole stalk. The affected region turns darker in color. Frequently there is a central black pipe running lengthwise of the stalk (Plate II, fig. 2). While it is not always possible to tell the presence of this fungus without a microscopical examination, a black discoloration is always suspicious. The intensity of this dark color will be increased if the stalk is cut open and laid in a moist place for twelve to twenty-four hours. The dark spores of the fungus develop very abundantly under such conditions.

This fungus does not seem to be very abundant in Louisiana. While it is occasionally found in south Louisiana, it does not have the same importance that it has in the tropics. Whether this is due to the fact that it has not become thoroughly distributed in this state or whether it is not adapted to our conditions is not at present known.

SPECIES OF FUSARIUM.

Fungi of the genus Fusarium are very common on seed cane in Louisiana. No attempt has been made to determine species in this genus but there are at least two distinct types of Fusaria present on the cane. These two types, for want of better names, are discussed in this bulletin under the names of purple Fusarium and white Fusarium. The purple Fusarium is a very large spored form which produces deep purple fruiting pustules on the cane and also produces the same colored growth on culture media. This fungus is commonly present on the outside of the stalks of seed cane during the late winter and is very easily recognized by the purple fruiting pustules.

The term, white Fusarium, as used in this bulletin, may include one or more species. The cultures which have been used in the investigations were quite similar and were closely related, if not identical with some of the ordinary Fusaria found on various decaying plant tissues. As these forms are all so similar, it seemed impossible for any one who has not made a particular study of Fusarium species to try to differentiate between the strains studied. In these forms, the mycelium is white in culture and the spores are of the common type, much smaller than those of the purple Fusarium.

The white Fusarium is present in almost every stalk of discolored seed cane. Its presence in a stalk can only be told by making cultures in the laboratory, yet it is a fact that it can be obtained from almost any discolored stalk.

SCOPULARIA SPECIES.

A species of Scopularia is found very commonly on the outside of stalks of seed cane in Louisiana. It forms patches of greyish mycelium on the stalks. If the stalks are in a humid place, the conidiophores of the fungus carrying little heads of spores develop very abundantly. Each little grey conidiophore bears a little glistening head of spores. This fungus may also occur on the inside of the stalk in split places, borer holes and other injuries.

On account of the frequent occurrence of this fungus on seed cane an attempt was made to identify it. A specimen was sent to the late Professor G. F. Atkinson of Cornell University, who said that it was probably an undescribed species of the genus Scopularia.

Inoculation Experiments.

In order to obtain some accurate data upon the effect of these organisms on planted cane, a series of inoculation experiments have been run during the past ten years. The mere presence of an organism, even if it is present on most of the stalks, does not necessarily mean that it is one of the factors in the poor germination of the eyes. Its real importance can only be ascertained by carrying on some controlled inoculation experiments.

It has been the aim in the experimental work to determine which organisms cause a decrease in the germination of the eyes and to determine the progress of the deterioration produced by each one. In order to obtain this information, inoculation experiments have been carried on under two different conditions. Canes have been inoculated with the several different organisms which seemed to be associated with seed deterioration and planted directly in the field. These tests are described as "Field Experiments." Then canes have been inoculated and placed in beds and covered with soil. These are described as "Bedding Experiments." This work has been conducted at the Sugar Experiment Station, Audubon Park, New Orleans, and at the State Station at Baton Rouge.

FIELD EXPERIMENTS.

In the field experiments, each test consisted of a series of plots. Generally a plot contained a single row two hundred and ten feet long, though in a few cases the plots were larger. In these plots was planted cane that had been treated or inoculated with different fungi. The plan of the tests was more or less the same in the different years in which the experiments were run. Check plots were used in which untreated seed was planted. The seed in the other plots was inoculated with pure cultures of the fungi. Generally the stalks were cut in two and the bottom halves were planted in one plot and the top halves in another. This was in order to test out the resistance of the hard ripened tissue at the bottom of the stalks with the soft unripened tissue of the tops.

The stalks were inoculated by three different methods. In some of the tests, the spores of the organisms from pure cultures were sprayed on the stalks after they had been laid in the furrows. In other tests, the stalks were inoculated by placing material from a pure culture in a puncture in the rind. A single puncture was made in each piece of cane. The pure culture was inserted into the stalk with one of the injectors used by veterinarians to inject blackleg pellets into an animal. A portion of pure culture was placed in the end of the needle and this was then pushed through the rind tissue and the culture left in the interior of the stalk. The hard tissue of the rind makes it impossible to use an ordinary needle with any satisfaction, especially if a large number of inoculations are required. Check punctures, in which no organism was used, were also made in the seed in some plots in order to determine the effect of the injury alone. Then in some of the tests, plots were planted with stalks that had been inoculated in the field during the preceding summer, several months before planting time.

In all of the experiments, cane was used just as it came from the field. The ideal way of performing an experiment of this kind would be to have the stalks perfectly free of the organisms which are liable to cause decay and then inoculate them with the ones desired. However, in practice, such a condition is absolutely impossible. These various fungi are present in the field on the stalks and it is impossible to sterilize them with any degree of satisfaction without damaging the eyes. Also the soil is more or less contaminated with the various fungi. In such experiments, then, as we have carried on, we must assume that the organisms are more or less present on all of the stalks but to a considerable greater degree on the inoculated ones. With such a condition, the results must be quite marked or else they are of little value.

The factors which are at work are so numerous and complex that a small variation of a few percent do not show anything. Furthermore, the results must be fairly consistent over a period of years.

In most of the tests carried on, counts were made of all the good eyes in each plot. These were usually made by the same person in order to eliminate as far as possible the personal error. While it is impossible to tell all the good eyes with certainty, especially when the eyes are being counted rapidly, this is possibly a more accurate and satisfactory method than that of measuring the combined lengths of the canes. In the experiments in which the eyes were not counted, the canes were laid end to end and were not overlapped, so that the combined length of the stalks planted in one plot was the same as in the others. In the tests in which the eyes were counted, the number of good eyes to the row ran from 325 to 700, depending upon the year, the variety of cane and the amount of seed put down. The stalks were usually overlapped to a considerable extent with the result that there was a considerable variation in the amount of cane planted in the different rows. The number of good eyes being known, this had no effect on the percentage of germination. Usually the number of good eyes to the row was between five and six hundred. In the other experiments in which the eyes were not counted, the stalks were laid end to end and the variation in the number of eyes was not so marked. Counts were always made of a few of the rows in order to get the average number of eyes.

The cane was planted in the plots in the same way as used by all planters in Louisiana. Two running stalks were planted in each furrow.

Effect of Puncture on Germination.

As many of the inoculations were made by puncture, it is first necessary to know what effect the puncture alone will have on germination. As the needle is liable to carry various organisms from the rind tissue to the inside, the puncture alone might be sufficient to cause some decrease in the germination. While the experiments to test this out were carried along at the time of the various inoculations, to save space in the tables the results are given by themselves. Two varieties of cane were used, D 74 and Louisiana Purple. The eyes were counted in each test. The results obtained during three years' trial are given in tables 2 and 3. The stalks were cut in two in the first two tests and the germination figured on both top and bottom halves.

TABLE 2.

Effect of puncture on germination percentage---Variety D 74.

YEAR	Bottom Halves		Top Halves		Whole Stalks	
	Check	Punctured	Check	Punctured	Check	Punctured
1914	25.2%	23.1%	17.7%	20.4%		
1915	18.7	20.9	11.1	8.6		
1916					9.4	12.0
Average	22.0	22.0	14.4	14.5		

TABLE 3.

Effect of puncture on germination percentage---Variety Purple.

YEAR	Bottom Halves		Top Halves		Whole Stalks	
	Check	Punctured	Check	Punctured	Check	Punctured
1914	9.9%	8.8%	12.7%	9.3%		
1915	22.3	20.7	16.3	14.4		
1916					14.5	10.0
Average	16.1	14.8	14.5	11.9		

An examination of the tables shows considerable variation in the different tests. Some tests showed a higher germination in the checks and some showed a lower. Averaging the whole set of ten tests, the checks show an average germination of 15.8% while the punctured stalks show an average of 14.8%. A variation of one percent in such an experiment comes well within the limits of the variation we might expect. While it is possible that puncturing may decrease the germination to a slight extent, this decrease is evidently not sufficient to account for the results obtained with some of the fungus inoculations to be discussed later.

Inoculations with Colletotrichum Falcatum.

As *Colletotrichum falcatum* seemed to be the most important of the organisms causing deterioration of seed cane in Louisiana, it has received the most attention. The inoculation and planting experiments under field conditions have been run over a period of seven years. The inoculations have been made mostly by puncture, though some tests have been made by spraying the spores directly on the canes in the furrows. The results of the puncture inoculations with D 74 and Purple cane are given in tables 4 and 5. Counts were made of the good eyes in all years excepting 1917.

TABLE 4.

Effect of puncture inoculations with Colletotrichum falcatum on germination percentage---Variety D 74.

YEAR	Bottom Halves		Top Halves		Whole Stalks	
	Check	Inoculated	Check	Inoculated	Check	Inoculated
1911	10.0%	5.0%				
1913	23.1	9.3	23.1	2.0		
1914	25.2	10.8	17.7	3.1		
1915	18.7	15.6	11.1	10.5		
1916					11.0	10.3
1917	12.2	3.0	6.5	1.3		
Average	17.8	8.7	14.6	4.2		

TABLE 5.

Effect of puncture inoculations with Colletotrichum falcatum on germination percentage---Variety Purple.

YEAR	Bottom Halves		Top Halves		Whole Stalks	
	Check	Inoculated	Check	Inoculated	Check	Inoculated
1913	21.8%	8.0%	10.4%	8.4%		
1914	9.9	5.6	12.7	6.0		
1915	22.3	16.2	16.3	11.4		
1916					14.5	10.6
1917	19.2	12.8	21.0	6.8		
Average	18.3	10.7	15.1	8.2		

An examination of the tables shows that the results obtained are remarkably consistent. In every case, the percentage of germination of the checks is above that of the inoculated and in most cases considerably above. Averaging the whole nineteen tests, it is found that the check stalks germinated 16.1% and the inoculated ones only 8.2%. Inoculating the stalks with *Colletotrichum falcatum* decreased the germination of the eyes nearly fifty percent. The tables also show that D 74 was injured more than the Purple and that the tops of D 74 were nearly ruined by the inoculation. The soft, unripened tissues of the tops seem to be less resistant than the ripened tissues at the bottom of the stalks.

During the years 1911, 1912 and 1916, some experiments were run in which the spores of *Colletotrichum falcatum* were sprayed upon the uninjured stalks of seed cane after they were laid in the furrows. In these tests four different varieties were used, D 74, D 95, Louisiana Striped and Louisiana Purple. In all, sixteen different tests were made. The stalks were cut in two and the bottom and top halves were tested separately. The results of these experiments were extremely variable. Out of the sixteen tests, eleven showed a higher germination in the checks and five showed a higher germination in the inoculated. Averaging all of the tests, the checks showed a germination of 20.8% and the inoculated a germination of 18.3%. While the inoculations may have decreased the germination to this slight extent, the difference is not sufficient to be conclusive. It is probable that there were spores of the red rot fungus on all the canes before they were planted. If it had been possible to use canes that were free of spores, probably more striking results would have been obtained.

As *Colletotrichum falcatum* causes a disease of the growing cane, it is evident that a considerable amount of cane affected with the red rot disease is used for planting. In order to find out what effect the presence of this disease in the stalks has upon germination, tests were run during four seasons. Enough stalks were inoculated in the field by puncture during July, August and September of these years to plant a plot along with the other inoculations. In 1913, 1914 and 1915, the stalks were cut in two and only the bottom halves were used for planting. This was necessary in order to have cane in the same condition as in the other plots. In 1916, the whole stalks were used, as whole stalks were used in all of the experiments of that season. As all of the inoculations in the growing cane were made in joints near the ground, the bottom halves showed most of the infection at planting time. The good eyes were counted in all of these tests. The results obtained in the different years are given in table 6.

TABLE 6.
Effect on the germination percentage caused by inoculating grown cane with Colletotrichum falcatum.

YEAR	D 74		PURPLE	
	Check	Inoculated	Check	Inoculated
1913	23.1%	18.8%	21.8%	15.8%
1914	25.2	18.7	9.9	25.3*
1915	18.7	22.9	22.3	21.2
1916	9.4	14.0	14.5	12.0
Average	19.1	18.6	17.1	18.6

*In 1914, the Purple cane that was inoculated in the field was from another plot than that from which the check cane was obtained, and it is evident that it was much stronger and better cane and superior for seed purposes.

Contrary to expectations, it was found that cane inoculated in the field with *Colletotrichum falcatum* did not show any marked decrease in germination. The average of the four years tests showed that it germinated practically as well as did the seed in the check plots. A possible reason for this will be considered on a later page.

Inoculations with Melanconium Sacchari.

The seed inoculation experiments with *Melanconium sacchari* have not been as extended as those with *Colletotrichum falcatum.* The field experiments with this fungus at Audubon Park were conducted during only two years, 1912 and 1914. Other than this, however, the bedding experiments have given considerable information regarding the effect of this fungus on planted cane.

The inoculation experiments at Audubon Park were carried on with two varieties, D 74 and Louisiana Purple. The stalks were cut in two and the top and bottom halves placed in separate plots. In 1912, the spores from pure cultures were sprayed on the stalks in the furrow, while in 1914 the stalks were inoculated by puncture. The results of the inoculations are given in table 7.

TABLE 7.
Effect of inoculations with Melanconium sacchari on germination percentage.

YEAR	Variety	How Inoculated	Bottom Halves		Top Halves	
			Check	Inoculated	Check	Inoculated
1912	D 74	Spray	36.0%	41.7%	21.0%	16.9%
1912	Purple	Spray	33.0	25.6	28.3	22.9
1914	D 74	Puncture	25.2	18.8	17.7	19.5
1914	Purple	Puncture	9.9	19.9	12.7	15.8

In the experiments at Audubon Park, inoculating the cane with *Melanconium sacchari* had very little effect on the germination of the eyes. Averaging all the results given in the table, it is found that the checks showed a germination of 23.0% and the inoculated cane a germination of 22.6%. The table also shows considerable variation in the results. In some cases the checks show a higher germination and in others a lower. From the results that have been obtained thus far, it can only be said that there is no evidence that *Melanconium sacchari* is of much importance in the deterioration of cane. It seems to attack dead stalks and rot the tissues rapidly but it does not seriously injure living cane. In fact, it may be of some importance in the soil in getting rid of the old stalks in the field.

Inoculations with Marasmius Plicatus.

Marasmius plicatus has never seemed to be a fungus that would cause a deterioration of the cane or one that would decrease germination to any extent. It develops on the outside of the stalks, between the leaf sheaths, around the buds and between the bud scales, but it does not enter the stalks except to follow in some injury such as a borer hole. Consequently this fungus has not been used in the field experiments to test out its effect on germination.

Experiments have been carried on at various times in the field to prove that the disease is carried over to new plants from the infected cuttings, both naturally and artificially infected. While these tests have shown that infected seed will produce infected plants, there was no evidence at any time that there was any important decrease in germination.

Inoculations with Gnomonia Iliau.

The experiments with *Gnomonia iliau* have been similar to those with *Marasmius plicatus.* No direct experiments have been carried on to determine the effect on germination. Many inoculations, however, have been made to determine whether the disease is carried over on the seed. In none of these experiments was there any marked decrease in germination. While the fungus would pass up to the young plant from the infected seed, it did not seem to kill the eyes before germination. Frequently when the infection was severe the young plants were killed when they were a few inches high, though generally the plants would live on through the season in a stunted, diseased condition.

Inoculations with Species of Fusarium.

Both the purple and white Fusariums have been used in the inoculation experiments to test out their effect on germination. With the purple one, inoculations were made both by puncture and by spraying the spores on the surface of the stalks. The results of the inoculations with this form are given in table 8.

TABLE 8.
Effect of inoculation with purple Fusarium on germination percentage.

YEAR	Variety	How Inoculated	Bottom Halves		Top Halves	
			Check	Inoculated	Check	Inoculated
1914	D 74	Puncture	25.2%	12.6%	17.7%	16.3%
1914	Purple	Puncture	9.9	13.9	12.7	14.6
1915	D 74	Spray	18.7	18.2		
1915	Purple	Spray	22.3	19.9		

With the purple Fusarium, the results obtained were variable. Sometimes the checks gave a higher germination and sometimes a lower. In only one case was there a marked decrease in the germination of the inoculated cane and consequently it seems likely that this result was due to some other factor. The experiments as a whole seem to show that this fungus is of minor importance in the deterioration of cane seed.

The experiments with the white Fusarium were only conducted during one season. In the fall of 1917, plots were inoculated by puncture with this fungus. Counts were not made of the good eyes but the stalks were laid end to end. The results obtained are given in table 9.

TABLE 9.
Effect of inoculation with white Fusarium on germination percentage.

YEAR	Variety	Bottom Halves		Top Halves	
		Check	Inoculated	Check	Inoculated
1917	D 74	12.2%	6.5%	6.5%	4.7%
1917	Purple	19.2	18.0	21.0	17.2

The results obtained with the white Fusarium, as far as they go, are perfectly consistent. In each test, the inoculated cane shows a lower germination than the check. However, it is impossible to draw conclusions from an experiment of this kind that has only been carried on for one year. Yet the results of these tests, coupled with the fact that this Fusarium is so extremely common in cane in all stages of deterioration, make it seem likely that this fungus is of some importance in decreasing the germination of the eyes.

Inoculations with Scopularia.

Inoculation experiments have been carried on during two years with the species of Scopularia that is so common on deteriorating cane. The results were variable and in no case was there any marked decrease in germination. Consequently it seems evident that this fungus is only of minor importance in the deterioration of seed cane.

BEDDING EXPERIMENTS.

Along with the field inoculation experiments that have been discussed above, bedding experiments were conducted during three seasons. In these experiments, good seed cane of the different varieties was obtained and divided into several piles. The canes in the different piles were then treated in different ways or inoculated with the different fungi. They were then placed in furrows and covered with a plow or hoe. There were from fifteen to forty stalks in each pile or bed. It was easy to open the beds and examine the canes in the spring or at various times during the winter and to study the effect of the inoculation or treatment. The inoculation experiments in the field only gave the actual effect on the germination percentage, while these tests gave information regarding the progress of the fungi in the stalks.

In most sections in the south, outside of the regular sugar belt, seed cane is carried over the winter in beds and then the good cane is selected out at planting time in the spring. In the sugar belt of Louisiana, this practice is not necessary as the temperature does not go low enough to hurt the cane planted in the field. Frequently in the sections where the cane is bedded, the loss of cane during the winter months is very high. The farmers say that the cane took the dry rot, or the wet rot or the red rot. This condition is not peculiar to Louisiana. The same condition exists in the syrup districts of Georgia. According to reports, the losses are sometimes severe in that state. Specimens which have been received from there show the same predominating fungi in the cane as are present in Louisiana. *Colletotrichum falcatum* appears to be the most common and destructive form present.

The bedding experiments to be discussed in this bulletin were carried on during three seasons, 1912, 1916 and 1917. In each case the tests were started in the fall and continued through the winter into the spring of the next year. The cane was bedded in the Experiment Station garden at Baton Rouge.

Experiments Started in 1912.

In the 1912 tests, the cane was treated and bedded on October 4. Four different varieties of cane were used, D 74, D 95, Louisiana Purple and Louisiana Striped. The D 95, Purple and Striped varieties were each divided into five piles of twenty to twenty-one stalks. One pile was left untreated as a check. The second pile was treated with benetol, 1-50, for about five minutes and the solution allowed to dry. The third pile was dipped in 4-4-50 Bordeaux Mixture and the solution allowed to dry. The fourth pile was sprayed with a suspension of spores of *Melanconium sacchari.* The fifth pile was sprayed with a suspension of spores of *Colletotrichum falcatum.* Not having enough of the D 74 cane to treat as above, it was divided into four piles of twenty stalks each. The first three piles received the same treatment as the first three piles of the other varieties, but the fourth pile was sprayed with a suspension of spores of both *Melanconium sacchari* and *Colletotrichum falcatum.* The good eyes were counted on all the stalks in each lot, the number of eyes running from 140 to 201 to the pile.

This cane was then covered and was not again disturbed until the first week of the following May when it was dug up and examined. At this examination the eyes that had germinated were counted and the germination percentage obtained. The results with the differently treated canes are given in table 10.

TABLE 10.

Germination percentage of bedded canes in 1912-13.

TREATMENT	D 74	D 95	Purple	Striped
Check	35.5%	11.0%	13.7%	22.6%
Benetol, 1-50	28.6	11.0	14.4	20.8
Bordeaux Mixture	26.6	6.5	14.6	29.1
Inoculated with *Melanconium sacchari*		16.2	22.5	21.0
Inoculated with *Colletotrichum falcatum*		5.7	10.7	14.6
Inoculated with *M sacchari* and *C. falcatum*	24.3			

The table shows a consistent decrease in germination in all the beds inoculated with *Colletotrichum falcatum* but variable results with *Melanconium sacchari.* This experiment alone would not be conclusive but in connection with the field experiments at Audubon Park, it helps to confirm the statement that *Colletotrichum falcatum* is responsible for considerable deterioration of seed cane.

All of the stalks were examined as they came out of the beds. The various fungi found on seed cane at that season of the year were present. *Colletotrichum falcatum, Melanconium sacchari,* and species of Fusarium and Scopularia were found in all of the beds and there was really little difference in the general appearance of the different lots of cane. There was also no material difference in the appearance of the split canes. They were all more or less reddened and soured. *Colletotrichum falcatum, Melanconium sacchari* and species of Fusarium were obtained from tissue cultures made from the interior of the stalks.

A series of cultures was also made from the dead eyes of a number of stalks from each pile. A single direct transfer was made from the interior of the eye or from the tissue at the base of the eye. After the cultures had developed, they were examined and the organisms present determined. The results are given in table 11.

TABLE 11.

Organisms in cultures made from dead eyes of bedded cane.

Treatment	Number of cultures made	ORGANISMS PRESENT				
		Bacteria	C. falcatum	M. sacchari	Fusarium	Other fungi
Check	40	40	4	0	23	5
Benetol, 1-50	42	42	4	0	25	7
Bordeaux Mixture	36	35	1	0	22	11
Inoculated with *M. sacchari*	32	31	2	4	16	7
Inoculated with *C. falcatum*	28	28	0	0	17	8
Inoculated with *M. sacchari* and *C. falcatum*	9	9	0	0	2	6
Total	187	185	11	4	105	44

The table shows that the predominating organisms in the dead buds of cane in the spring are species of bacteria and Fusarium. It is reasonable to expect that the eyes at that time would be filled with various saprophytic forms. There was very little difference in the fungous flora of the buds from the different beds.

Experiments Started in 1916 and 1917.

In the years 1916 and 1917, the bedding experiments were carried on in a somewhat different manner. Instead of spraying spores on the stalks in the beds, each stalk was inoculated by puncture. Then at frequent intervals throughout the winter, some of the stalks were dug up, cut open and examined. While this procedure prevented the obtaining of any germination percentage, it permitted a study of the activities of the different forms in the cane. In both years only one variety, the Louisiana Purple, was used.

In 1916 the seed cane was placed in eight beds with forty stalks to the bed. One bed was used as a check, the stalks being punctured but not inoculated. The other seven beds were inoculated by puncture with the following organisms: *Colletotrichum falcatum, Melanconium sacchari, Gnomonia iliau, Marasmius plicatus, Scopularia sp.,* purple Fusarium and some bacterial forms that had been found abundant in deteriorating cane.

In 1917, the seed cane was placed in eight beds with twenty stalks to the bed. In one bed, the stalks were punctured but not inoculated; in a second bed, the stalks were inoculated with *Colletotrichum falcatum;* in a third bed, the stalks were inoculated with the white Fusarium; and in a fourth bed, the stalks were inoculated with both the Colletotrichum and the Fusarium. The stalks in the other four beds were soaked in formaldehyde solution, 1-100, for forty minutes and then treated in the same manner as the stalks in the first four beds. As the formaldehyde did not seem to have any particular effect, this really made forty stalks with each treatment.

The cane was examined every week or ten days and some stalks from each bed were split open. Careful notes were taken on the appearance of the cane and a series of photographs was made to show the gradual progress of the rots that developed.

The check stalks developed a certain amount of discoloration in the punctured joints. The tissue would first become somewhat reddened around the puncture and this discoloration would gradually extend back through that joint. Any injury in cane is followed by the development of a red color. The injury allows the entrance of various yeasts and bacteria which set up a fermentation. This discoloration, however, did not usually pass through the node except in a few cases where *Colletotrichum falcatum* happened to gain entrance accidentally. Late in the winter and early spring, many of the check stalks became more or less reddened throughout but this was due to the entrance of various fermentation forms which had gained entrance to the interior at various points in the rind tissue. These points of entrance could usually be readily told. In cane planted in the field, it is difficult to find a stalk in the early spring that is not more or less reddened. In the experiments with bedded cane, general reddening did not develop from the point of puncture in the check stalks.

The stalks inoculated with *Melanconium sacchari,* purple Fusarium, *Gnomonia iliau, Marasmius plicatus, Scopularia sp.* and the bacteria developed very much as did the check stalks. There was nothing to show that any of these forms had attacked the cane to any extent. Rarely did the discoloration in the inoculated joint pass through the nodal tissue. While the discoloration possibly developed slightly faster in the inoculated cane than it did in the check, by the end of the winter there was no difference in the appearance of the stalks. In Plate VI are shown stalks inoculated with these forms as compared to the check.

The effect of *Colletotrichum falcatum* in seed cane, however, was perceptible very shortly after the inoculation. The fungus grew rapidly through the internodes and was able to pass easily through the nodal tissue. A week after the inoculation, the fungus had invaded three joints of the stalk, the inoculated and the two adjoining ones. After this period the development of the rot was somewhat slower. This may have been partially due to the cooling of the soil caused by the approach of the winter season, though perhaps other factors may also have been present. However, there was a gradual development of the rot, until by spring usually the greater part of the stalk showed the presence of the fungus. It was also noticed that as the fungus approached the end joints of the stalk, its development was much impeded. Frequently the end joints of the stalk would not be infected with the fungus. This was probably due to the counteracting effect of other organisms such as bacteria and yeast which had entered through the cut ends. On account of this, frequently the inoculated stalks would only show developing buds on the two end joints. This same condition has also been noted in the fields.

The development of *Colletotrichum falcatum* in seed cane inoculated at planting time is somewhat different than its development in growing, living stalks. The appearance of red rot in growing stalks is very characteristic (Plate IV, fig. 1, and Plate V, figs. 1 and 2). The fungus grows rapidly through the fibrovascular bundles, causing them to change to a deep red in color. At places, the fungus will break out of these bundles and spread out through the other tissues. These areas are at first turned red in color but later transverse white spots appear in the red tissue. The red fibrovascular strands and the peculiar white spots in the red areas are very characteristic of the red rot disease in growing cane. In the growing stalks, also, the spread of the disease through the stalk is rather slow. With the varieties commonly grown in Louisiana, it takes several months for the fungus to spread through four or five joints of the stalk (9).

Seed cane that was inoculated at planting did not develop the characteristic red bundles or the definite white spots that are so common in growing cane affected with the red rot disease. While red bundles were sometimes present they were also often absent. The fungus was not held in the fibrovascular bundles; it apparently was able to grow rapidly through any of the tissues of the cane stalk. The affected regions first became slightly reddened or else had a watersoaked appearance. Later the affected areas took on various shades of red, brown, grey and white. After the tissues had been diseased for sometime, they usually became brownish or else greyish white. The colors were so extremely variable that it is impossible to state any definite characteristic. Frequently, also, there were small black areas in the nodes and sometimes in the bundles of the internodes. The nodal tissue always became diseased either changing to a brown or a greyish white. The buds were killed in nearly all cases. Typical stalks at different stages in the development of the fungus are shown in Plates VIII and IX.

It is difficult to explain the difference in development of *Colletotrichum falcatum* in growing stalks and in seed cane. It may be that growing cane is able to produce some counteracting substance which tends to keep the fungus confined to the fibrovascular bundles and to keep it from spreading rapidly in the other tissues of the stalk. There is a development of gum around the infected areas in growing stalks but it is not known whether this tends to check the development of the fungus or not. If the growing cane is able to produce some counteracting substance which cannot be produced by the cut cane, this would explain the variation in the development of the fungus. This would also account for the fact that cane inoculated some time previous to the cutting did not deteriorate when used as seed as rapidly as the stalks that were inoculated after cutting.

The white Fusarium did not seem to develop rapidly when alone. It spread quickly through the inoculated internode but was not able to pass readily through the nodal tissue, though in time it did pass into the adjoining joints. This fungus colored the internal tissues of the stalk a deep red. Red fibrovascular bundles also were fairly abundant. Stalks inoculated with this fungus are shown in Plate IX, figures 1a and 2a. When double inoculations were made with this fungus and *Colletotrichum* *falcatum,* the Fusarium could usually be reisolated from the affected joints.

Cultures were readily obtained from the stalks inoculated with *Colletotrichum falcatum* and the white Fusarium. Direct transfers were usually made from the stalks at each examination in order to make certain that the fungi were developing and were responsible for the deterioration.

The results obtained from the bedding experiments are in perfect agreement with the field tests. Both series of experiments show that *Colletotrichum falcatum* attacks the seed cane and causes a rapid deterioration accompanied by a poor germination of the buds. Furthermore it would seem from the experiments conducted that it is the chief seed destroying fungus at present in Louisiana. While there may be many factors at work in cane deterioration, there is little question but that *Colletotrichum falcatum* is responsible for a considerable portion of the loss.

TEMPERATURE RELATION.

The relation of temperature to the organisms which occur on seed cane is of considerable interest. The cane is usually planted while the ground is still warm, but during most of the winter months while the cane is lying dormant the temperature of the soil is comparatively low. It is interesting to compare the temperature of the soil during the different months with the rate of growth of the fungi at different temperatures.

The rate of growth of three of the fungi at different temperatures was determined. The organisms were grown in petri dishes in an incubator which could be regulated for different temperatures. A single large batch of bean pod agar was made and used in all of the plates in order that there would be no variation in the medium. The organisms used in the test were *Colletotrichum falcatum, Marasmius plicatus* and *Gnomonia iliau.* While the two latter forms do not seem to be responsible for much of the seed deterioration, they are important because they live over winter on the seed cane and attack the young shoots in the spring. The rate of growth was determined by measuring the increase in diameter of the colonies and finding the average daily increase over a period of four or five days. In figure 1 are shown graphically the results of this test. The curves show the average daily growth in millimeters at the different temperatures. The temperatures at which the cultures were grown were 13°, 18.5°, 22.5°, 24°, 27°, 35° and 37.5°C.

The fungi were able to grow at all of the temperatures tried but the optimum for all was somewhere in the neighborhood of 27°C. At higher or lower temperatures, the growth was not as rapid. *Colletotrichum falcatum* and *Marasmius plicatus* seemed to grow well at any temperature between 20° and 35°C, but the latter temperature seemed to be too high for *Gnomonia iliau.*

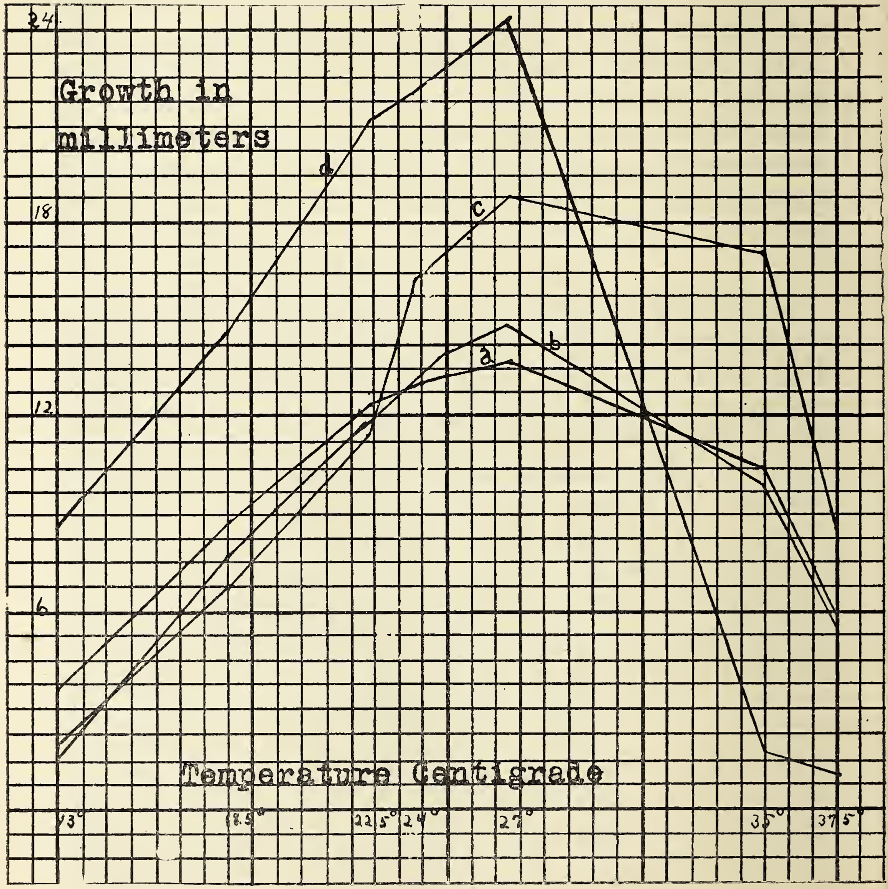

Fig. 1. Curves showing rate of growth of different fungi at different temperatures. *a* and *b*. Two different cultures of *Colletotrichum falcatum*. *c*. *Marasmius plicatus*. *d*. *Gnomonia iliau*.

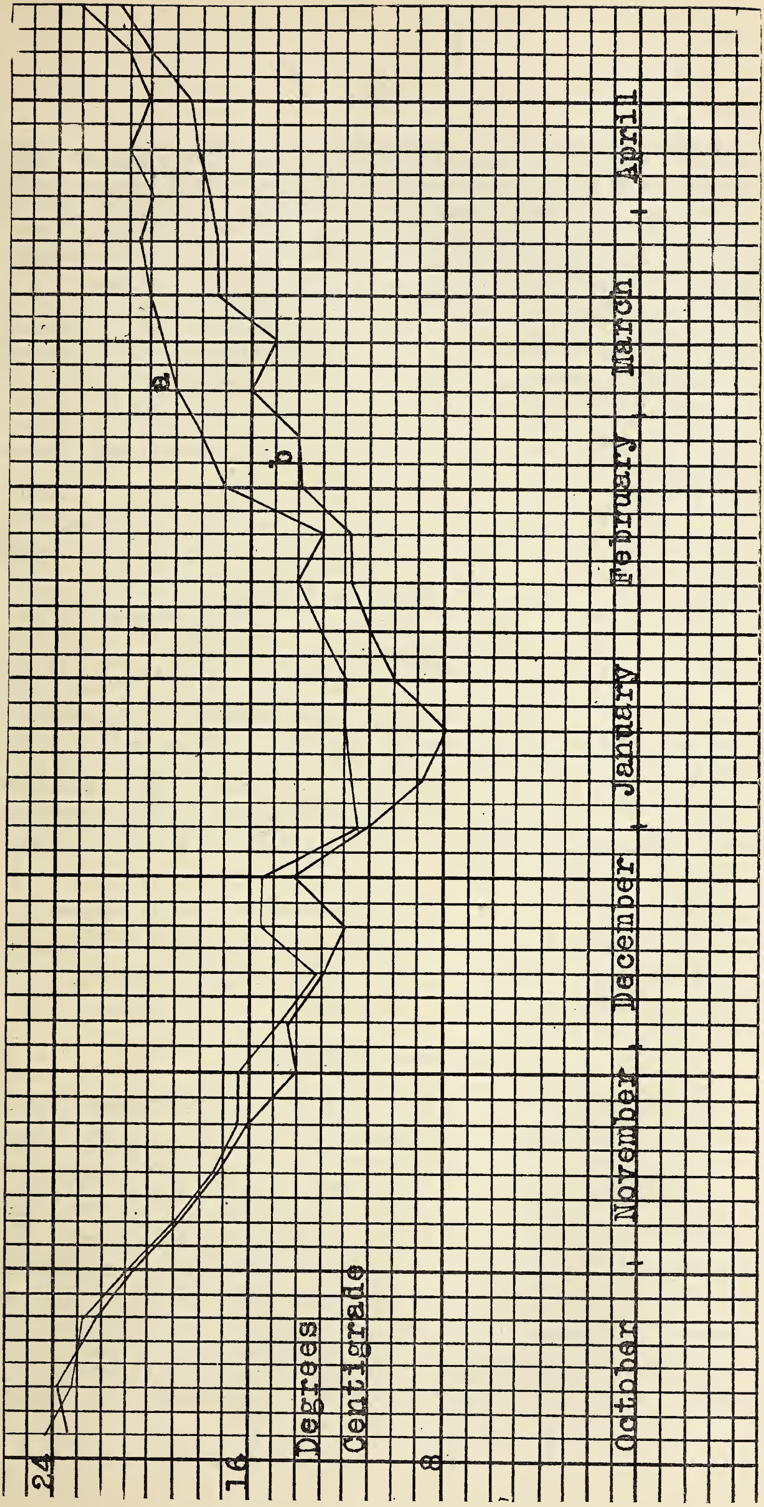

Fig. 2 Mean soil temperatures, average of two and a half years. *a*. Audubon Park, New Orleans, La. *b*. Experiment Station, Baton Rouge, La.

In order to determine the temperature of the soil in which the cane was planted or bedded, a soil thermograph was located in the field at Audubon Park, New Orleans, and one in the Experiment Station garden at Baton Rouge. These automatically recorded the soil temperatures at a depth of three inches. These instruments have been in operation over two complete seasons and a part of a third. In figure 2 are charted curves showing the average weekly mean temperature of the soil at the two stations for this period. The weekly mean temperature was obtained by averaging the highest and lowest recorded temperatures for the week.

The curves show that the temperature of the soil drops rapidly after the middle of October and that for a period of two or three months during the winter, the temperature is too low for a satisfactory development of the fungi. A suitable temperature for the development of the organisms is not reached until nearly the first of March. As a matter of fact, seed cane ordinarily shows the greatest deterioration during the late winter and early spring. The red rot fungus will develop rapidly in the stalk during the fall if it gains entrance at planting time but it seems probable that most of the natural infection takes place slowly during the winter months and then a rapid development occurs with the approach of warm weather.

The effect of cold on the development of *Colletotrichum falcatum* was easily seen in the inoculated stalks of the bedded cane. During weeks of severe weather there seemed to be but little change in the appearance of the cane.

TREATMENT OF SEED CANE.

As most of the organisms which cause deterioration of seed cane are on the stalks at planting time, it would seem that something might be accomplished by treating the seed with some disinfectant before planting. Experiments along this line have been carried on at many of the tropical experiment stations with varied results. In a recent bulletin, Johnston (16) has compiled the results which have been obtained in the various countries. Conditions in most of the tropical countries are somewhat different than in Louisiana. Most of the experiments in these countries have had the primary object of controlling the pineapple disease fungus, *Thielaviopsis paradoxa,* a fungus which seems to be of only minor importance in Louisiana at present.

In Louisiana, seed treatment experiments have been conducted during two seasons. The disinfectants which were used were formaldehyde and corrosive sublimate. The formaldehyde solution was made by mixing one pint of commercial formaldehyde with thirty gallons of water, the same proportion as is used in the ordinary potato scab treatment. This solution was placed in tanks and the seed cane was immersed in it for thirty to forty-five minutes. The cane was planted without drying. The corrosive sublimate solution was made to the strength of 1-1000. It was made with the ordinary commercial antiseptic tablets, one tablet being added to each pint of water. The seed cane was placed in the solution for five minutes and then taken out and planted without washing. As corrosive sublimate is a powerful antiseptic and will remain after drying, there appeared to be no necessity for a longer treatment.

The first test of these solutions was made in the fall of 1917. Two varieties of cane were used and the top and bottom halves of the stalks were planted separately. On June 1, 1918, the stalks that had grown were counted and the percentage of germination was obtained. The results are given in table 12.

TABLE 12.

Effect of disinfectants on the germination of cane, 1917-18.

TREATMENT	D 74		PURPLE	
	Bottom Halves	Top Halves	Bottom Halves	Top Halves
Check, no treatment	12.2%	6.5%	19.2%	21.0%
Formaldehyde, 45 minutes	15.2	10.2	29.5	23.7
Corrosive sublimate, 5 minutes	15.3	14.7	29.7	21.5

As is shown in the table, in 1917 there was an increased germination in all of the plots that were planted with treated cane. Further than this, the field examination that was made of the plots showed that the plants which grew from the treated canes, especially those treated with corrosive sublimate, were more vigorous and grew off faster than those in the untreated plots.

In the fall of 1918, the experiment was again tried but on a larger scale. Three varieties, Purple, D 74 and L 511, were used and six to nine rows were planted in each plot. Also in this experiment whole stalks were used for planting. The corrosive sublimate treatment was used with all varieties but the formaldehyde was used with only the L 511 cane. After the cane had all germinated in the following spring, the stalks were counted and the percentage of germination was obtained. The results of the test are given in table 13.

TABLE 13.

Effect of disinfectants on the germination of cane, 1918-19.

TREATMENT	D 74	Purple	L 511
Check, no treatment	19.7%	27.0%	25.6%
Formaldehyde			21.5
Corrosive sublimate	17.9	25.6	28.6

In the 1918 test, as is shown in the table, the germination percentage did not seem to be materially affected by the treatment. In three cases, the check plot showed a higher germination and in one case a lower, but in no case was the variation very marked.

The germination percentage, however, does not tell the whole story of this experiment. Notes were also taken on the vigor of the developing stalks and at the end of the season, the tonnage, sucrose content and other data were obtained.

Shortly after the plots came to a stand in the spring, a marked difference was noted between the rows of L 511 and Purple cane that were treated with corrosive sublimate as compared to those not treated. The stalks from the treated seed were larger and had a healthier appearance than those from the untreated seed. With the L 511 variety, this difference was apparent all through the summer. At the last examination shortly before the grinding season, the plants in the treated plots were taller and the stalks were considerably larger and better developed. With the Purple variety, as the season advanced, the difference became less noticeable until at grinding time the rows in the untreated and treated plots seemed to be about the same. The plots of D 74 cane did not at any time show the variation in vigor as was noticed with the other varieties.

Why the different varieties did not show the same variation in vigor is not perfectly clear. It is possible, however, that the Mosaic disease had something to do with this. Both the Louisiana Purple and the D 74 varieties showed practically 100% infection with this disease and as this trouble seems to have a tendency to check growth of the canes, especially the older ones, its presence may partially explain the variation noted with the different varieties. The D 74 cane, especially, seemed to be seriously injured by the Mosaic. The L 511 cane is somewhat resistant to the Mosaic disease and does not seem to be seriously affected by the trouble.

The soil conditions in the treated and untreated plots were as nearly identical, as far as is known, as it is possible to have them. The plots were side by side.

In November, during the grinding season, the plots of the L 511 and the Purple varieties were cut, weighed and ground and the number of stalks counted. The D 74 cane was used for seed purposes and the data was not obtained for this variety. The data obtained with the first two varieties is given in table 14.

TABLE 14.

Effect of disinfectants on the yield, etc., of cane, 1918-19.

Variety	TREATMENT	Stalks per acre	Tons per acre	Percent sucrose
L 511	Check	15,481	9.316	14.28
L 511	Corrosive sublimate	16,885	13.729	14.14
L 511	Formaldehyde	19,353	11.430	14.29
Purple	Check	18,109	12.242	10.98
Purple	Corrosive sublimate	19,496	12.535	10.71

As is shown in the table, the data obtained during the grinding season bear out the general observations made during the growing season. With the L 511 cane, the yield on the formaldehyde treated plot was over two tons greater than on the check and on the corrosive sublimate treated plot over four tons greater. On the latter plot, the increase in tonnage was practically fifty percent. With the Purple cane, the corrosive sublimate treated plot with a slightly lower germination percentage gave a slightly greater tonnage. There were but small differences in the analyses of the canes on the different plots.

SUMMARY.

The principal points discussed in this study of the effect of fungi on the germination of sugar cane, a project which has been in progress for a period of ten years, are summarized below.

1. Sugar cane does not germinate as well in Louisiana as it does in the tropics, the average germination being about 20%. This necessitates heavy planting and consequently materially increases the cost of the crop.

2. The buds, or eyes, on cane tops do not germinate quite as well as do those on the bottom halves of the stalks. This seems to be due to the more rapid deterioration of the soft tissues of the cane tops.

3. A great many factors are instrumental in the deterioration of seed cane but one of the important ones is the action of certain parasitic fungi.

4. A great many different fungi are found on seed cane but the more abundant or more serious ones are *Colletotrichum falcatum, Melanconium sacchari, Gnomonia iliau, Marasmius plicatus, Thielaviopsis paradoxa* and species of *Fusarium* and *Scopularia.*

5. *Colletotrichum falcatum,* which also causes the disease of growing cane known as the red rot, is very common on seed cane and produces a serious deterioration. The germination percentage of the eyes of stalks inoculated by puncture with this fungus at planting time is reduced about 50%. The natural method of infection in such cane seems to be through injuries and through the root buds around the nodes.

6. *Colletotrichum falcatum* develops in cane that has been inoculated after cutting more rapidly than it does in growing cane. Also the infected stalks have a somewhat different appearance.

7. Stalks that have a good infection of red rot before cutting do not deteriorate when used as seed as rapidly as stalks that are inoculated after cutting.

8. In Louisiana, there is no evidence that *Colletotrichum falcatum* passes up into the young stalks from the diseased seed.

9. *Melanconium sacchari* is very abundant on deteriorating cane but does not seem to decrease the germination percentage.

10. *Marasmius plicatus* and *Gnomonia iliau* occur on seed but

there is no evidence that they reduce the germination percentage. They do, however, pass up on to the young stalks from the seed.

11. *Thielaviopsis paradoxa* occurs very sparingly in Louisiana and as yet does not seem to be responsible for much deterioration.

12. Two types of Fusarium are abundant on seed cane. The purple species does not seem to be important in the deterioration of seed. The white species may reduce the germination percentage to some extent.

13. A species of Scopularia is abundant on seed cane but is of little economic importance.

14. The optimum temperature for growth of several of the fungi that were studied is around 27°C. Most of the forms make but slow growth at the normal temperature of the soil during the winter season.

15. Seed cane has been treated at planting time with formaldehyde and corrosive sublimate. While the tests have not been continued long enough to be conclusive, the results are encouraging. In the best test, a 50% increase in tonnage was obtained from a plot treated with corrosive sublimate.

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EXPLANATION OF PLATES.

Plate I. The ordinary appearance of deteriorated or dead seed cane during the late winter and spring season.

Plate II. Fig. 1. A cane stalk infected with *Gnomonia iliau.* Fig. 2. A stalk infected with *Thielaviopsis paradoxa.*

Plate III. Fig. 1. Cane stalks infected with *Melanconium sacchari.* Fig. 2. Split stalks of seed cane infected with *Colletotrichum falcatum.* These stalks were placed in a moist chamber for a few days in order for the fungus to develop some mycelium.

Plate IV. Fig. 1. A split stalk of growing cane infected with *Colletotrichum falcatum.* Fig. 2. A stalk of sugar cane infected with *Marasmius plicatus.*

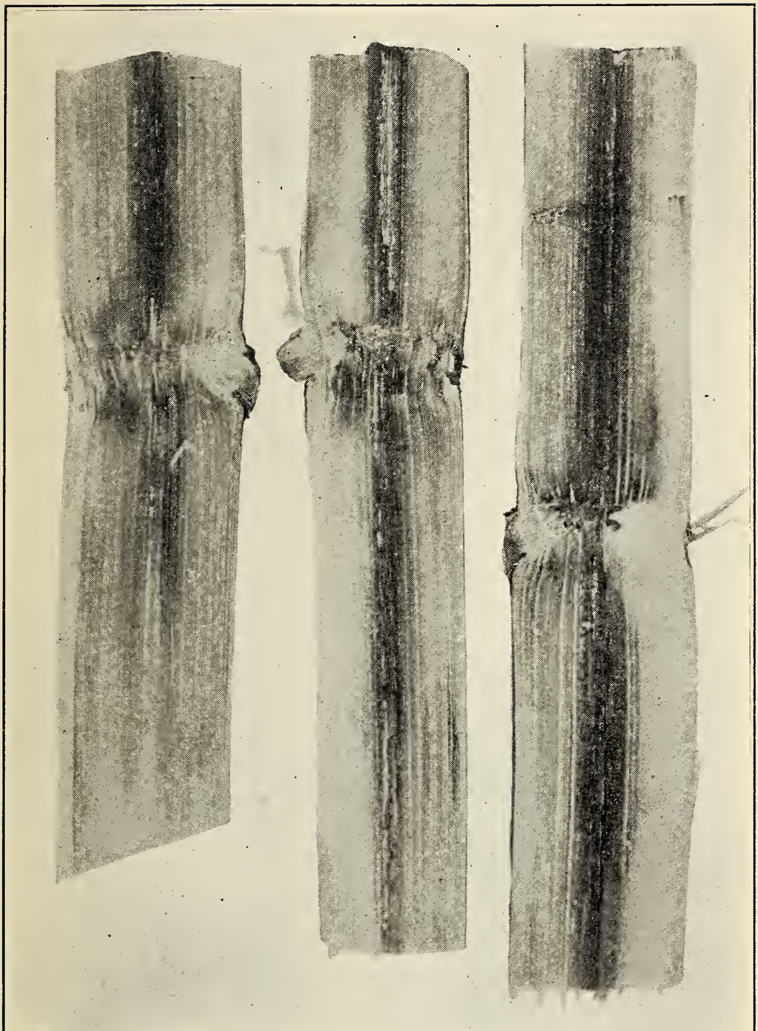
Plate V. Stalks of growing cane artificially inoculated with *Colletotrichum falcatum.* Fig. 1. A very old infection. Fig. 2. A younger infection.

Plate VI. Seed cane inoculated at planting time with different organisms. Stalks photographed during the winter season. Fig. a. Check, stalk punctured but not inoculated. Fig. b. *Melanconium sacchari.* Fig. c. *Scopularia sp.* Fig. d. Purple *Fusarium.* Fig. e. *Marasmius plicatus.* Fig. f. *Gnomonia iliau.* Fig. g. Species of bacteria.

Plate VII. Effect of planting cane inoculated with *Colletotrichum falcatum.* Two rows on the left of the center, not inoculated; two rows on the right of the center, inoculated.

Plate VIII. Development of *Colletotrichum falcatum* in inoculated seed cane. Fig. 1a. Check, stalk not inoculated but punctured. Fig. 1b. Inoculated stalk eight days after inoculation. Fig. 2. Inoculated stalks three weeks after inoculation.

Plate IX. Development of *Colletotrichum falcatum* and the white Fusarium in inoculated seed cane. Fig. 1. Three weeks after inoculation. Fig. 2. Two months after inoculation. *c.* Check stalk, not inoculated. *b.* Stalks inoculated with *Colletotrichum falcatum.* *a.* Stalks inoculated with the white Fusarium.



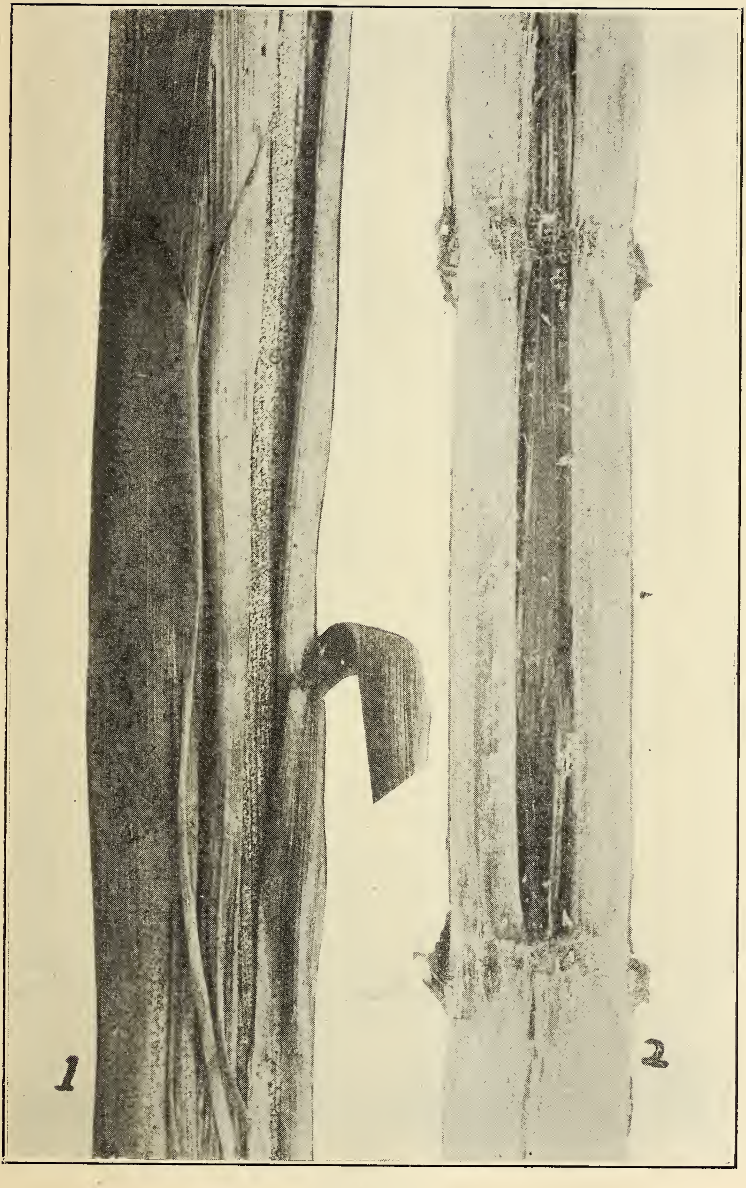
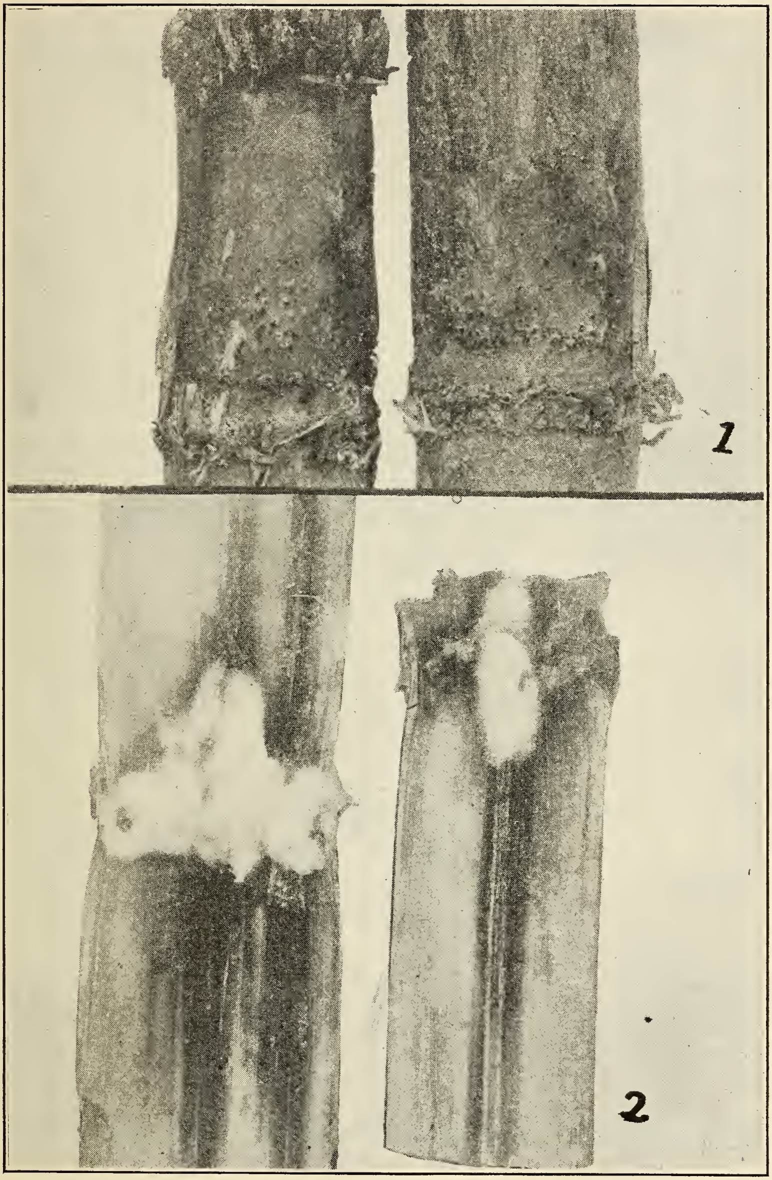


PLATE II.



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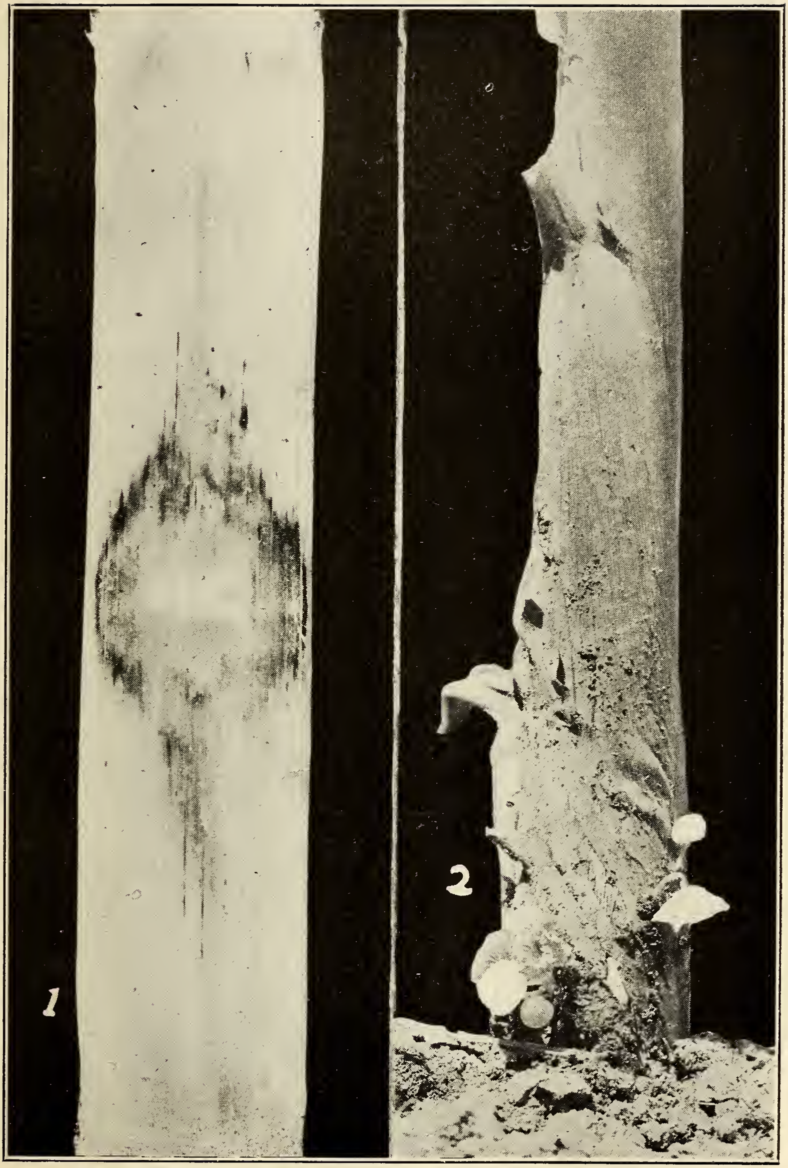

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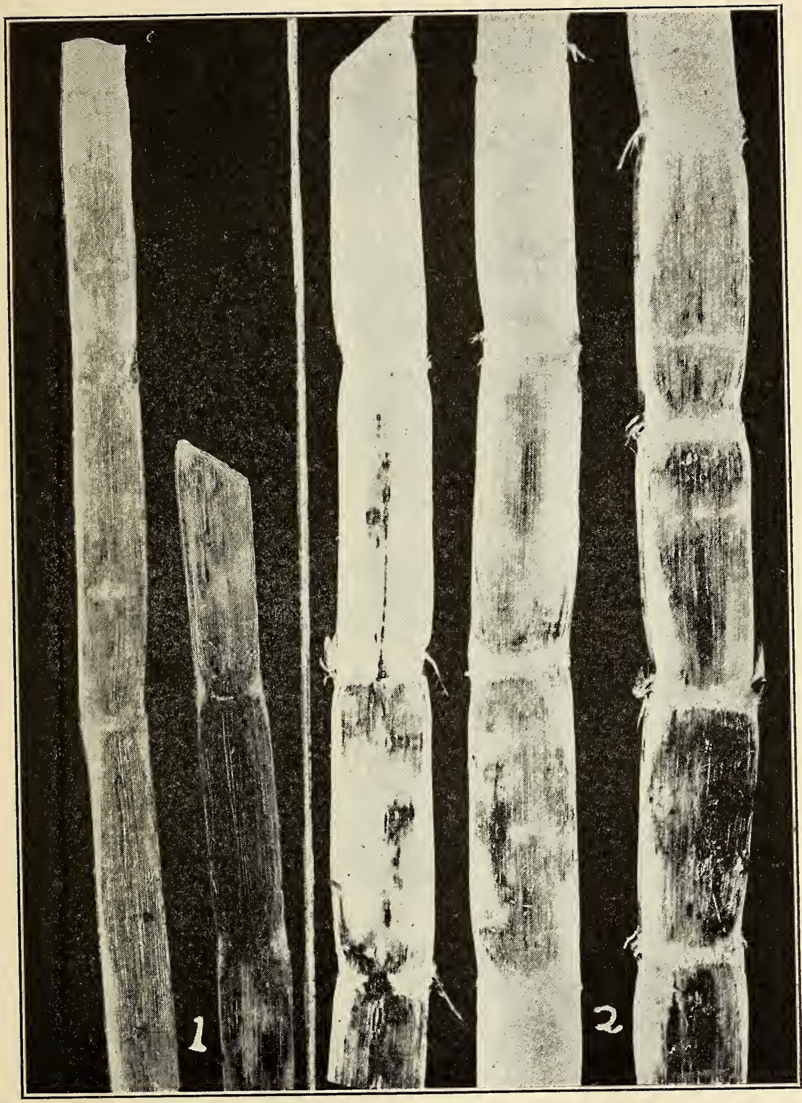

PLATE V.

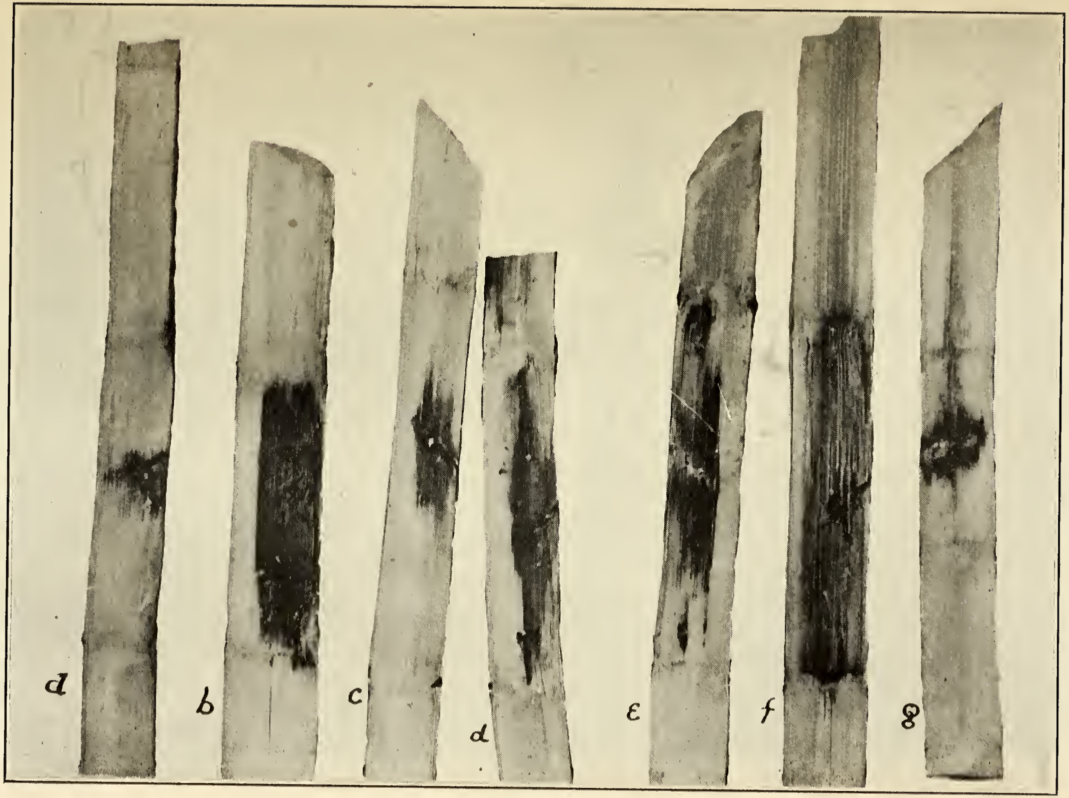

PLATE VI.

PLATE VII.

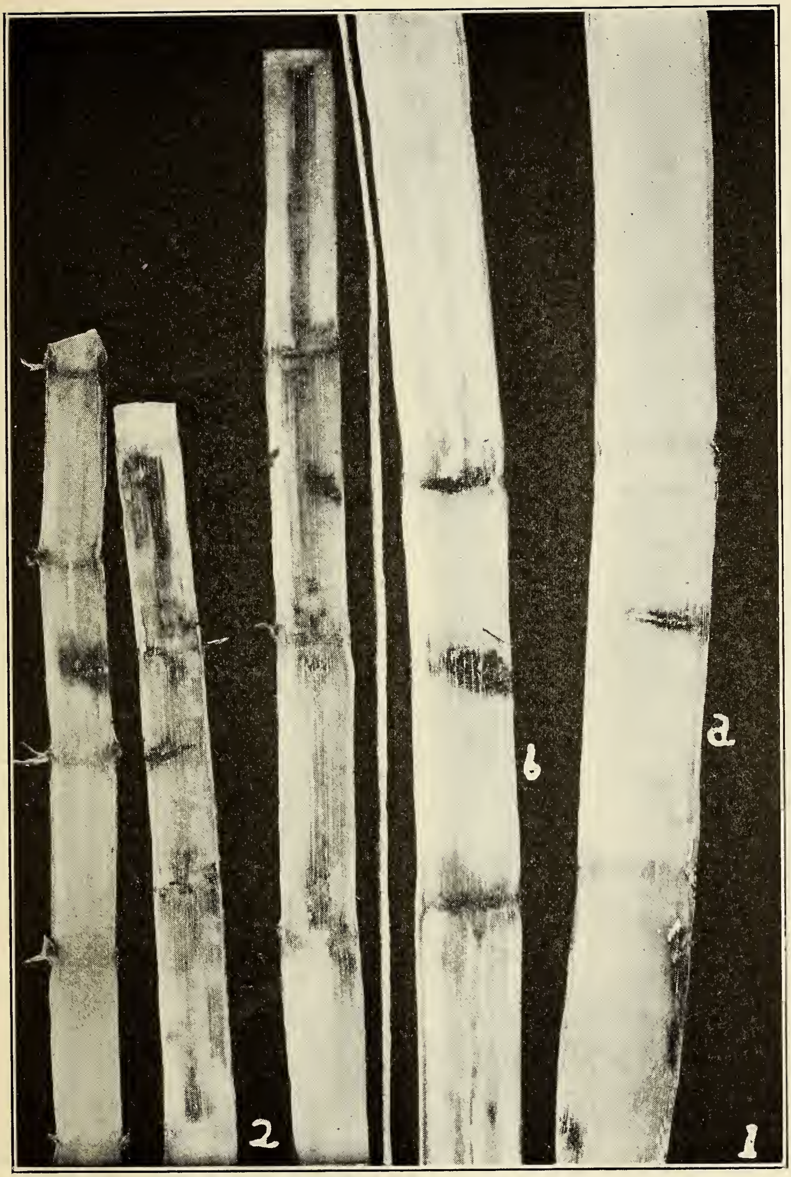

PLATE VIII.

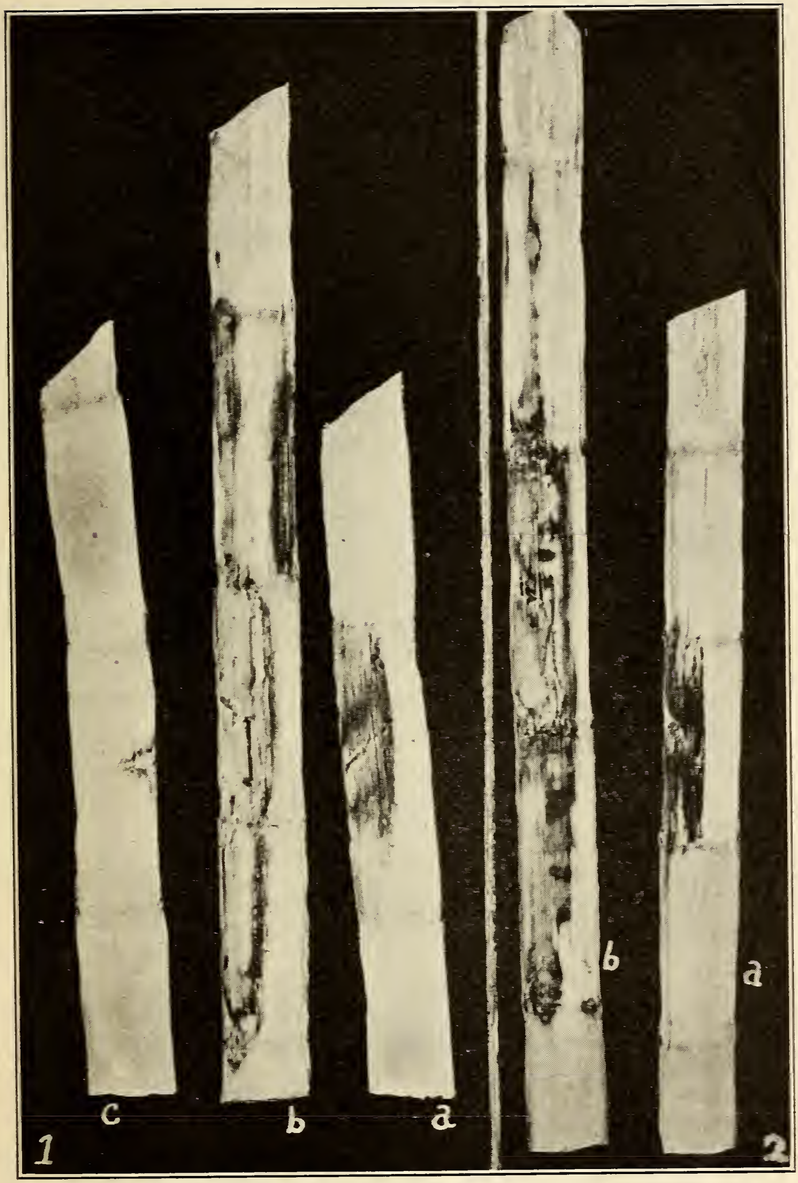

PLATE IX.

Louisiana Bulletin No. 170 *July, 1920*

Agricultural Experiment Station

of the

Louisiana State University and
A. & M. College
Baton Rouge

FACTORS DETERMINING THE KEEPING QUALITY OF CANE SUGAR.

(WITH A CHART FOR PREDICTION)

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Bacteriologist

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GLADNEY'S PRINT SHOP BATON ROUGE, LA.

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Factors Determining the Keeping Quality of Cane Sugar.

(With a Chart for Prediction)

Submitted for Publication December 13, 1919.

By

NICHOLAS KOPELOFF and LILLIAN KOPELOFF,

Bacteriologist and Assistant Bacteriologist.

INTRODUCTION

The problem of sugar deterioration is not so much a matter of scientific concern as one which commands the attention of sugar planters, manufacturers, refiners and dealers. There exists at present an unanimity of opinion in ascribing the phenomenon of sugar deterioration to the activities of microorganisms, and it is unnecessary at this point to discuss the valuable contributions in which the evidence has been advanced, because they have been considered in detail in a previous publication.† Suffice it to say, that heretofore the bacteria received most attention as causative agents in sugar deterioration. However, in our investigations we have been concerned chiefly with molds. In a bulletin entitled "The Deterioration of Cane Sugar by Fungi,"† there was included an exhaustive survey of the kinds of molds occurring in a variety of marketable cane sugars. It was found that there were three molds which occurred universally in the sugars examined, namely, *Aspergillus Sydowi* Bainier, *Aspergillus niger*, and *Cladosporium*, which are frequently found in soil, vegetable products of all kinds, water, etc. These molds were examined for their power to deteriorate sugar by inoculating sterile sugars with them and analyzing before and after incubation. It was found that these molds were capable of inducing very serious losses of sucrose in a very short time, if the conditions for their activity were favorable. However, deterioration also occurred where the moisture conditions were unfavorable. Where mycelia developed, deterioration occurred, for it is axiomatic that enzymes are liberated during cellular activity, and the physical evidence of the growth or cellular activity of molds is to be found in the mycelial stage. On the other hand, it was puzzling indeed to find evidence of enzyme activity where the molds were unable to grow. This led to a closer scrutiny of the inoculating material, which consisted of mold spores, and raised the question, do mold spores contain enzymes, which represents the point of departure for the ensuing investigations.

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†See literature cited on page 64.

Part I.

DO MOLD SPORES CONTAIN ENZYMES*

Molds have long been known to produce intracellular and extracellular enzymes, but so far as we have been able to ascertain no study has been made of the enzymes contained in the spores of these organisms. This is indeed noteworthy since the valuable contributions of Duclaux, Fernbach and other mentioned by Effront[3] and Dox[2] have apparently been concerned solely with the enzymic activities of molds in the mycelial stage. It is our purpose to limit this discussion to the invertase activity of the spores of *Aspergillus niger,* because of the economic significance of this enzyme and the wide distribution of the mold in nature.

The method of procedure was as follows:

A large number of Petri dishes containing Kopeloff's agar[5] were seeded with a single pure culture of *Aspergillus niger* and incubated at 35°C for 6 days. A small amount of sterile distilled water was introduced into each plate, successively, shaken slightly and then poured on sterilized filter paper (Whatman No. 4). The mycelium was then washed through the filter paper with sterile distilled water, which of course was responsible for the loss of a large number of the spores. However, a separation between the mycelium and spores was thus effected by a combination of filtration and flotation, and it only remained to continue the process long enough to accumulate a desired quantity of spores, and which served to remove any invertase which may have been excreted by the mycelia. These spores were then rinsed off the filter paper into a sterile Erlenmeyer flask (300 cc.). To 250 cc. of this spore suspension and an equal volume of sterile water was added 1 cc. of c. p. chloroform. The flasks were heated to 63°C for 30 minutes for the purpose of killing the spores[15] and making the spore wall more permeable. The contents of each flask were transferred to Erlenmeyer flasks containing sterile washed sand and shaken vigorously for 5 minutes in an atttempt to further rupture the spore walls. The inoculum was then ready to be added to the 200 cc. portions of 10 per cent. (by weight) granulated sugar solution in cotton plugged Erlenmeyer flasks which had been previously sterilized in the autoclave at 15 lbs. pressure for 15 minutes, all the flasks having the same polarization. Ten cc. of sterile distilled water were added to the check flasks, while 10 cc. and 20 cc. of inoculum were added to the others. Following this inoculation both the inoculum and sterile distilled water were heated to 100°C for 20 minutes to kill any enzyme which might be present. Then this sterilized inoculum was added to the sugar solution as outlined above. All flasks were then incubated at 35°-40°C for 3 hours. At the end of this time 50cc. of solution were removed with a sterile pipette, filtered and polarized.* It may be noted parenthetically, that we have recorded the polarization values and the average decrease in actual per cent. sucrose, calculated therefrom, in separate columns. Reducing sugars were determined in the same sample by the modified Violette method (volumetric). The results are recorded in Table 1. Only the averages of closely agreeing triplicate determinations are presented in the following tables.

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* Read by title at Buffalo Meeting of the American Chemical Society, April, 1919.

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*The authors wish to thank Mr. E. C. Freeland, Assistant Chemist, for his assistance with these analyses.

TABLE 1

Analyses of 10 per cent. Sugar Solutions Inoculated with Spores of *Aspergillus niger*.

Av.	TREATMENT	Single Pol.	% Decrease in pol.	% Decrease in Sucrose	% Reducing Sugars	*Probable Error	% Increase in Reducing Sugars
	3 HOURS INCUBATION						
10 cc.	Sterile H_2O heated 63°C.	35.6			.04	.012	
10 cc.	Sterile H_2O heated 100°C.	35.8			.04	.0	
10 cc.	Spores heated 100°C.	35.7			.04	.0	
10 cc.	Spores heated 63°C.	34.8	0.9	.23	.06	.002	.02
20 cc.	Spores heated 63°C.	32.1	3.6	.90	.07	.001	.03
	Corrected to 10 cc.	34.1	1.5	.38	.07		.03
	24 HOURS INCUBATION						
10 cc.	Sterile H_2O heated 63°C.	35.6			.05	.0	
10 cc.	Sterile H_2O heated 100°C.	35.9			.04	.0	
10 cc.	Spores heated 100°C.	35.8			.04	.0	
10 cc.	Spores heated 63°C.	31.0	4.6	1.16	.20	.0	.15
20 cc.	Spores heated 63°C.	26.0	9.6	2.41	.48	.016	.43
	Corrected to 10 cc.	27.2	8.4	2.11	.50		.45
	4 DAYS INCUBATION						
10 cc.	Sterile H_2O heated 63°C.	37.3			.05	.0	
10 cc.	Sterile H_2O heated 100°C.	36.8	0.5	.12	.04	.0	
10 cc.	Spores heated 100°C.	36.9	0.4	.09	.04	.0	
10 cc.	Spores heated 63°C.	24.1	13.2	3.31	.93	.026	.88
20 cc.	Spores heated 63°C.	18.1	19.2	4.80	2.00	.106	1.95
	Corrected to 10 cc.	18.9	18.4	4.55	2.09		2.04

*In this and all succeeding tables P. E. is understood to be a + or — value.

It will be seen from the results in Table 1 that the flasks inoculated with sterile distilled water and those inoculated with sterilized inoculum (heated to 100°C) had the same polarization, 35.7 (within experimental error) and the same amount of reducing sugars, .04, while the flasks having 10 cc. of spores heated to 63°C polarized 34.8 which represents a loss in polarization of 0.9 as compared with the check, or a decrease of .23 per cent. sucrose. The reducing sugars increased .02 per cent. The inoculum of spores was polarized alone and exhibited no optical activity. However, where 20 cc. of spores heated to 63°C were used the polarization dropped to 34.1 which was a decrease of 1.5 per cent as compared with the check, or an actual decrease of .38 per cent. sucrose. The reducing sugars were slightly increased. Where 20 cc. of inoculum were used it is necessary to correct for the added dilution, if we wish to compare these results with those obtained with 10 cc. of inoculum. Furthermore, it must be remembered that throughout these results each polarization value represents the optical activity of all three sugars present, namely, sucrose, dextrose, and levulose, and consequently cannot be accepted unreservedly as an adequate criterion of inversion; rather one must consider the reducing sugars for such a purpose. From these results it is evident that after 3 hours the enzymic activity of the spores of *Aspergillus niger* is already manifest. A microscopic examination of the contents of each flask was made for the purpose of detecting any germination if present. No germination occurred. Thus the enzymic activity as evidenced by the reduction in polarization and increase in reducing sugars, was produced by the spores of the mold present. That this inversion was truly enzymic is proven by the fact that when the spores were heated to 100°C, which temperature kills all enzyme activity, the inoculation induced no change in the composition of the original sugar solution.

The flasks each received 3 drops of chloroform and were returned to the incubator at 45°C. where they remained until analyzed at the end of the 24 hour incubation period. It may be mentioned in passing that the flasks were frequently shaken to assist in the rupturing of the spore walls. From the results recorded in the second part of Table 1 it will be noted that, as previously, the flasks inoculated with the sterile water heated to 63°C. and 100°C. as well as the sterilized inoculum (spores heated to 100°C.) polarized about 35.8 and contained about .04 per cent. reducing sugars. On the other hand, there is a marked decrease in polarization in the flasks inoculated with 10 cc. of spores heated to 63°C. as shown by the value 31.0, representing a loss of 4.6 or a decrease of 1.16 per cent. sucrose. The reducing sugars increased appreciably to .15 per cent. from .05 per cent. Still more striking is the fact that double this quantity of spores (i.e. 20 cc.) was responsible for a proportional decrease in polarization (amounting to 8.4 or a decrease of 2.11 per cent. sucrose as compared with the check), and a slightly greater increase in reducing sugars. Again, as a result of a microscopic examination, no signs of germination could be detected. Thus the results after 24 hours confirmed those obtained after 3 hours incubation, and we must conclude that the spores of *Aspergillus niger* have released sufficient of the enzyme, invertase to carry forward the inversion of sucrose already noted.

Finally, after adding 3 drops of chloroform and again incubating, the flasks were analyzed at the end of 4 days. The results are to be found in the third part of Table 1. Compared with the previous analyses the check flasks, i.e. sterile water heated to 63°C. and 100°C. and spores heated to 100°C. show a slightly higher and somewhat less consistent polarization. This is undoubtedly due to evaporation, the flasks having been plugged with cotton. The discrepancies are negligible, as evidenced by the uniformity in amount of reducing sugars present, which represent the true criterion of inversion. Where 10cc. of spores heated to 63°C. were used, the polarization is reduced to 24.1 as compared with 37.3 in the check, a loss of 13.2 or a decrease of 3.31 per cent. sucrose. The reducing sugars indicate a correspondingly high increase of .88 per cent. Where 20 cc. of inoculum were used, it will be observed that the polarization is reduced 18.4, as compared with the check, or a decrease of 4.55 per cent. sucrose, and the reducing sugars increased to 2.04 per cent. Again no germination could be detected in a microscopic examination. Therefore, there can be no doubt that it has been demonstrated that the spores of molds contain enzymes—as exemplified by the release of invertase by the spores of *Aspergillus niger*.

Since the particular problem which gave rise to the present investigation, namely, the deterioration of sugar, is concerned primarily with solutions of high concentration, it was planned to corroborate the above evidence by repeating the experiment and using a sugar solution of 20 per cent., or double the concentration of that previously employed. The methods and technique were identical with those described in the experiment just reported, except that in this case there were somewhat fewer spores present in the inoculum, and each flask contained 200 cc. of a 20 per cent. (by weight) sugar solution.

TABLE 2

Analyses of 20 Per Cent. Sugar Solutions Inoculatedwith Spores of *Aspergillus niger.*

24 HOURS INCUBATION

Av. TREATMENT	Single Pol.	% Decrease in Pol.	% Decrease in Sucrose.	% Reducing Sugars.	Probable Error	% Increase in Reducing Sugars.
10 cc. Sterile H_2O heated 63°C.	61.0			.05	.002	
10 cc. Sterile H_2O heated 100°C.	61.2			.05	.0	
10 cc. Spores heated 100°C.	62.2			.05	.0	
10 cc. Spores heated 63°C.	58.0	3.0	.72	.38	.008	.33
20 cc. Spores heated 63°C.	50.5	10.5	2.52	1.16	.064	1.11
Corrected to 10 cc.	52.9	8.1	1.94	1.21		1.16

In Table 2 are recorded the results of the analyses after 24 hours incubation at 45°C. It will be observed that the polarization and reducing sugars of the flasks receiving 10 cc. of sterile distilled water heated to 63°C. and those heated to 100°C. were the same. Ten cc. of spores heated to 100°C. gave a slightly higher polarization, but these variations are probably the result of the sterilization process which did not affect all flasks in an identical manner. However, there is no question concerning the reduction in polarization as effected by 10 cc. of spores heated to 63°C. and which amounts to 3.0 or a decrease of .72 per cent. sucrose. The reducing sugars show a somewhat greater increase, namely, .33 per cent. Where 20 cc. of spores were employed the polarization is 52.9, as compared with 61.0 in the check flasks, a reduction of 8.1, or a decrease of 1.94 per cent. sucrose. Correspondingly there is an increase in reducing sugars of 1.16 per cent. No germination could be detected as a result of a microscopic examination. Thus the results obtained with a 20 per cent. sugar solution confirmed those previously obtained with the 10 per cent. solution and form an adequate basis for establishing the fact that the spores of *Aspergillus niger* contain invertase.

TABLE 3

Summary of Enzymic Activity of Spores of *Aspergillus niger*.

Spores	Concentration of Sugar Solution	Incubation Period	Decrease in polarization.	Decrease Actual Per Cent Sucrose.	Decrease in per cent. Sucrose Compared with Original as 100.	Per cent. Increase in Reducing Sugars.	Per cent. Increase in Reducing Sugars Compared with Original as 100.
10	10%	3 hrs.	0.9	.23	2.5	.02	50
10	10 do.	24 do.	4.6	1.16	13.0	.15	300
10	20 do.	24 do.	3.0	.72	4.9	.33	660
10	10 do.	96 do.	13.2	3.31	35.4	.88	1760
20*	10 do.	3 do.	1.5	.38	4.2	.03	75
20*	10 do.	24 do.	8.4	2.11	21.9	.45	900
20*	20 do.	24 do.	8.1	1.94	13.3	1.16	2320
20*	10 do.	96 do.	18.4	4.55	50.0	2.04	4080

*Corrected to 10 cc.

In Table 3 the data previously presented are summarized in such a way as to make evident the correlations which are of interest. For example, it will be readily observed that with a given quantity of inoculum in a sugar solution of definite concentration, there is a progressive decrease in polarization accompanied by an increase in reducing sugars with an increase in the incubation period. Again it may be noted that increasing the inoculum causes practically a proportional decrease in polarization and a corresponding increase in reducing sugars. This lends further support to the evidence brought forward that mold spores contain enzymes, for it is precisely under such circumstances that a relationship is established between that number of spores and the amount of enzyme activity. While the data presented do not establish such a correlation with mathematical accuracy, one may assume that the number of spores whose walls become ruptured would be somewhat variable in quantity and consequently a progressive increase in enzymic activity may be seen to accompany an increase in the number of spores.

It is especially interesting to note that 20 cc. of spores reduced the polarization of a 10 per cent. sugar solution in 4 days in such a way as to indicate the loss of 50 per cent. of the original sucrose present. This inoculum accounted for the loss of 22 per cent. of the original sucrose in 24 hours. The increase in reducing sugars is even more striking in intensity as seen in the last column of Table 5, where in the cases mentioned above, the amounts were increased 4080 and 900 per cent. respectively, compared with the original taken as 100.

When the inoculation of a 10 per cent. solution with 10 cc. of spores is compared with a similar inoculation of a 20 per cent. sugar solution, it will be seen that in 24 hours the amount of reducing sugars in the latter case is about double that in the less concentrated solution. A similar phenomenon is to be observed when 20 cc. of spores are employed. This is in line with the theoretical considerations of the activity of the enzyme invertase and indicates that the amount of inversion depends upon the quantity of sucrose present. The polarization values do not permit of such clearly defined generalizations, because of the more complex nature of the factors involved.

It is irrelevant at the present time to do more than indicate the significance of the industrial applications of this biological principle. We have already mentioned that it plays a considerable role in the deterioration of sugar by materially affecting the factor-of-safety rule. It may, likewise, explain certain transformations in the soil which occur as a result of mold activity where mycelia are not found in great abundance. Thus the universal distribution of molds and their activities will undoubtedly suggest many further developments of this phenomenon, some of which are at present under investigation in this laboratory.

SUMMARY

1. The spores of *Aspergillus niger* heated to 63°C. for 30 minutes and shaken with sterile sand, caused a decrease in polarization and an increase in reducing sugars in a 10 per cent. sterile solution in 3 hours, and continued the same changes throughout the 4-day incubation at 45°C. These results were corroborated when a 20 per cent. sugar solution was similarly inoculated.

2. Spores heated to 100°C. caused no change (neither did an inoculation with sterile distilled water) proving that the activity mentioned above was enzymic in nature.

3. The enzyme present exhibited properties identical with invertase, consequently the spores of *Aspergillus niger* contain invertase.

4. Among the practical applications of this phenomenon the deterioration of manufactured cane sugar and certain transformations in the soil are especially significant.

Part II.

THE INVERTASE ACTIVITY OF MOLD SPORES AS AFFECTED BY CONCENTRATION AND AMOUNT OF INOCULUM.*

It has been previously shown[6] that mold spores may release sufficient invertase to cause an appreciable inversion in 10 and 20 per cent. sucrose solutions. This phenomenon was studied in connection with the deterioration of cane sugar by fungi[5], and in considering its bearing upon the latter problem it becomes imperative to establish the limits of concentration at which the invertase of mold spores is active[6]. The work of O'Sullivan and Thompson[10] is responsible for the claim that invertase is not active in sucrose solutions above 40 per cent. We have been unable to find in the literature any contrary stand, although there exists some indirect evidence on this point. Since the film surrounding the sugar crystal capable of supporting biologic activity must vary in concentration from a supersaturated solution down to comparatively low concentrations (depending upon the amount and distribution of the moisture present), it was considered advisable to employ concentrations of 10, 20, 30, 40, 50, 60 and 70 per cent. at an incubation temperature of 48°-50°C. for three days. The spore suspensions were prepared according to the method previously described[6]. The solutions were all examined microscopically at the end of the incubation period, and no germination occurred; sterility was determined by plating on Kopeloff's agar.

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*We are indebted to Mr. S. Byall for his assistance with the chemical analyses.

TABLE 4

Influence of Concentration on Invertase Activity of Spores of *Aspergillus Sydowi* Bainier*.

CONCENTRATION	Pol.	Loss in Pol.	Loss Per Cent. Sucrose.	Reducing Sugars Per Cent.	Gain Reducing Sugars Per Cent.
10 Per Cent Check	44.2			0.03	
10 Do. Heated Spores	44.0	0.2		0.03	
10 Do. Spores	54.1	5.9	1.5	1.79	1.76
20 Do. Check	85.1			0.07	
20 Do. Spores	71.0	13.2	3.2	2.78	2.71
30 Do. Check	124.6			0.12	
30 Do. Spores	107.3	17.3	4.0	2.78	2.66
40 Do. Check	166.6			0.14	
40 Do. Spores	147.7	18.9	4.2	2.94	2.00
50 Do. Check	52.8			1.00	
50 Do. Spores	47.4	5.4	5.4	4.41	3.41
60 Do. Check	57.7			1.71	
60 Do. Spores	54.6	5.1	3.1	4.17	2.46
70 Do. Check	66.1			2.13	
70 Do. Spores	61.5	4.6	4.6	4.54	2.41

*144,000 Spores per cc. 10-40 per cent.
100,000 Do. Do. 50-70 per cent.

In Table 4 are presented the averages of closely agreeing triplicate determinations obtained with the spores of *Aspergillus Sydowi* Bainier* at the rate of 144,000 per cc. for 10 to 20 per cent. solutions of pure sucrose, and 130,000 spores per cc. for 50 to 70 per cent. It will be noted that with an increase in concentration up to 60 per cent. there is a proportional loss in per cent. sucrose and corresponding increase in reducing sugars. Beyond this point the amount of inversion decreases. This brings out two important facts, namely, that the invertase in the spores of this mold is active in a sugar solution at the point of saturation, and furthermore that the maximum activity occurs between 50 and 60 per cent concentration for such an inoculation. It may be mentioned, parenthetically, that the polarization of 10-40 per cent. solutions was made directly (using 100 mm. observation tube where necessary), while 26 gms. in 100 cc. were polarized in the 50-70 per cent. solutions. Consequently, the conversion of polarization to per cent. sucrose in the 40 per cent. solutions is a calculation based upon an assumed Brix and specific gravity.

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*The tentative identification of this organism as "Blue Aspergillus" now becomes "Aspergillus Sydowi Bainier", according to Dr. Charles Thom's identification corroborated by the describer of the species.

TABLE 5

Influence of Concentration on Invertase Activity of *Penicillium expansum.*

CONCENTRATION	Pol.	Loss in Pol.	Loss Per Cent. Sucrose.	Reducing Sugars Per Cent.	Gain Reducing Sugars Per Cent.
10 Per Cent Check	40.0			0.03	
10 Do. Heated Spores	40.0			0.03	
10 Do. Spores	24.8	15.2	3.8	2.77	2.74
20 Do. Check	82.6			.17	
20 Do. Spores	58.3	24.2	5.8	4.17	4.00
30 Do. Check	122.6			.21	
30 Do. Spores	95.3	27.3	6.4	5.00	4.79
40 Do. Check	170.7			.23	
40 Do. Spores	128.2	42.5	9.5	5.77	5.24
50 Do. Check	53.0			.97	
50 Do. Spores	49.9	3.1	3.1	2.87	1.90
60 Do. Check	67.7			1.71	
60 Do. Spores	57.6	0.1	0.1	2.62	0.92
70 Do. Check	66.1			2.13	
70 Do. Spores	64.6	1.6	1.6	2.73	0.60

In Table 5 are shown the results similarly obtained using the spores of *Penicillium expansum.* It is to be regretted that the inoculations differed so widely, for there were 5,000,000 spores per cc. in the 10-40 per cent. solutions, and only 220,000 spores per cc. in the 50-70 per cent. solutions. However, it is evident that with an increase in concentration up to 50 per cent. there is a marked loss in sucrose accompanied by a corresponding increase in reducing sugars. Beyond this point the loss in sucrose is not so significant, the same being true of the gain in reducing sugars. One may be permitted the speculation that had the inoculation in 50-70 per cent. solutions been 25 times larger, the results would have corroborated those previously obtained with *Aspergillus Sydowi* Bainier, namely, that the invertase released by the spores of *Penicillium expansum* was active in concentrations of sugar as high as the saturation point and attained a maximum between 50 and 60 per cent. concentration.

TABLE 6

Influence of Concentration on the Invertase Activity of the Spores of *Aspergillus niger.*

CONCENTRATION	Pol.	Loss in Pol.	Loss Per Cent. Sucrose.	Reducing Sugars Per Cent.	Gain Reducing Sugars Per Cent.
50 Per Cent Check	52.0			1.10	
50 Do. Heated Spores	52.0			1.40	0.24
50 Do. Spores	40.6	11.4	11.4	6.94	5.54
60 Do. Check	57.7			1.71	
60 Do. Spores	48.1	9.6	9.6	6.67	4.96
70 Do. Check	66.1			2.13	
70 Do. Spores	67.0	7.1	7.1	5.18	3.06

In Table 6 are recorded the results obtained with the *Aspergillus niger* in concentrations above 50 per cent. sucrose, since we have previously indicated an increase in invertase activity of the spores of this organism with an increase in concentration[6]. It will be seen that the per cent. sucrose lost and reducing sugars gained at 50 per cent. show a maximum, and that there is a diminished but significant activity up to the saturation point as in the case of the *Aspergillus Sydowi* Bainier and *Penicillium expansum.*

While it is indeed difficult to draw any comparative conclusions concerning the invertase activity of the three molds under consideration because of the difference in the number of spores used for inoculation, nevertheless it can safely be postulated that the spores of the *Aspergillus Sydowi* Bainier are relatively more active in inverting power than the other two molds employed. For it will be seen that with 130,000 spores of this mold per cc. at the highest concentration employed (which was at the saturation point) there was a gain of 2.41 per cent. in reducing sugars (over the check) while with double the number of spores of *Penicillium expansum* there was only one-fourth that gain in reducing sugars. Five times as many spores of *Aspergillus niger* produced one-fourth greater increase in reducing sugars.

In general, then, it will be seen that the invertase activity of these mold spores is directly influenced by concentration in the manner described, and furthermore that it is likewise dependent on the number of spores employed. The second part of this paper, therefore, is concerned with the influence of numbers of mold spores upon their invertase activity in sugar solutions at the saturation point.

Since there has been occasion in another connection[6] to study the influence of this factor, somewhat the same procedure was employed. The spore suspensions were prepared as mentioned[6] and the proper dilutions made with sterile distilled water. The same conditions of incubation, etc., were again observed. The check flasks contained a mixture of spores and all dilutions heated 100°C. for ½ hour. The largest number of spores in the case of *Penicillium expansum* and *Aspergillus niger* was about 400,000 per cc., and 80,000 with *Aspergillus Sydowi* Bainier, the dilutions representing an arithmetic progression of one-half of the preceding quantity.

TABLE 7

The Influence of Numbers of Spores on the Invertase Activity of Mold Spores in 70 Per Cent. Sugar Solutions.

No. of Spores Per 1 cc. Soln.	Per Cent Sucrose (Pol.)	Loss in Per Cent Sucrose.	Per Cent Reducing Sugars.	Gain in Per Cent. Reducing Sugars.
ASPERGILLUS SYDOWI BAINIER				
Check	65.5		.97	
80,000	59.7	6.8	4.55	3.86
40,000	63.1	3.4	2.78	1.81
20,000	64.7	1.8	2.10	1.21
10,000	63.0	1.5	1.50	0.53
5,000	65.3	1.0	1.07	0.10
PENICILLIUM EXPANSUM				
Check	66.5		1.50	
440,000	61.1	5.2	3.57	2.07
220,000	63.8	2.5	2.67	1.17
110,000	64.9	1.4	1.90	0.40
55,000	65.2	1.1	1.48	0.
28,000	65.1	0.2	1.48	0.
ASPERGILLUS NIGER				
Check	66.4		0.81	
400,000	64.0	2.4	1.31	0.50
200,000	65.3	1.1	1.00	0.19
100,000	66.5		1.07	0.26
50,000	66.3	0.1	0.86	0.05
25,000	66.3	0.1	0.88	0.07

In Table 7 will be found results obtained with such varying quantities of inoculum in sugar solution at the point of saturation. Some striking differences are to be noted where *Aspergillus Sydowi* Bainier was used. Thus with a decrease in size of inoculum there is a proportional decrease in invertase activity until 5,000 spores are employed where no significant inversion occurs. Consequently that number may be taken as the lowest limit essential to invertase activity in such a highly concentrated sugar solution.

An interesting feature of this series of experiments was that *Aspergillus Sydowi* Bainier secreted an enzyme which caused a formation of gum in the sterile sugar solutions. The amount of gum formed was proportional to the number of spores per gm. employed. The properties of this gum and the conditions under which it is formed are now being investigated.

On the other hand *Penicillium expansum* is not nearly so effective, for it will be seen that while there is a decrease in invertase activity with a decrease in number of spores, nevertheless, the minimum is reached between 110,000 and 55,000 per cc., which is practically ten times as great an amount as was required by the *Aspergillus Sydowi* Bainier. The lower limits of the invertase activity of *Penicillium expansum,* however are very clearly defined. The results obtained with *Aspergillus niger* are practically identical, qualitatively, with those observed in the case of *Penicillium expansum,* but vary in the quanitative relationships. To a certain degree this is probably accounted for by the fact that the latter mold had the larger inoculation.

While the limitations of the data herein presented are clearly recognized in so far as it is exceedingly hazardous to state the limits of enzymic activity in terms of such units, because of the variations which must exist between different strains of the same species, nevertheless, the results are suggestive in their bearing upon the practical problem of sugar deterioration. A certain correlation to be emphasized is that the *Aspergillus Sydowi* Bainier which was found to have the greatest capacity for deteriorating sugar (as well as occurring with greatest frequency) has spores which appear to exhibit the most intense invertase activity in saturated sugar solutions. Since we have pointed out that sugar inoculated with spores of this mold deteriorated without the development of mycelia, the inference established would appear to be substantiated, namely, that mold spores alone, if present in sufficient number, are capable of deteriorating sugar.

SUMMARY.

1. The invertase activity of the spores of *Aspergillus Sydowi* Bainier, *Aspergillus niger* and *Penicillium expansum* is exhibited at concentrations of sugar varying from 10 to 70 per cent.

2. The maximum invertase activity of these mold spores occurs between 50 and 60 per cent. concentrations.

3. An increase in the number of mold spores is responsible for increased invertase activity in a saturated sugar solution.

4. The least number of spores per cc. of *Penicillium expansum* and *Aspergillus niger* required to produce inversion in saturated sugar solution is between 50,000 and 110,000. About 5,000 spores of *Aspergillus Sydowi* Bainier are needed to cause inversion.

5. The evidence that mold spores alone are capable of deteriorating cane sugar is corroborated by the data herein presented.

Part III.

THE EFFECT OF CONCENTRATION ON THE DETERIORATIVE ACTIVITY OF MOLD SPORES IN SUGAR.*†

It is common knowledge that the reduction of the moisture content of sugar is responsible for a diminuition of deterioration. This is tantamount to stating that an increase in concentration of the films surrounding the individual particles in any given mass of sugar produces the same effect. The influence of this factor on the activities of bacteria has been the subject of thorough investigation by Owen (11, 12, 13, 14), and our recent work (5) with molds has led to the conclusion that "the factor of safety for sugars well infected with molds would appear to be lower than is generally supposed". The full import of such a consideration depends to a considerable degree upon the fact that molds may be responsible for the inversion of sucrose where only spores are present as well as when mycelia were developed. Furthermore, it has been shown that the invertase activity of mold spores is exhibited at concentrations of sugar solutions varying from 10 to 70 per cent. with maximum activity at 50 to 60 per cent. In the same connection it was observed that an increase in the number of mold spores is responsible for increased invertase activity at any definite concentration, including saturated solutions. While these might be considered sufficient grounds for inference regarding the deteriorative activity of mold spores in sugars of known composition, it was deemed advisable to carry out such an investigation on a more practical scale (8).

With this end in view, a series of sugars with films of known composition were made in the laboratory by coating large crystals of sterilized sugar with sterilized blackstrap molasses and 60° Brix sugar syrup in definite proportions, and purging in the centrifugal, a method previously employed with success (5). These sugars and corresponding molasses were aseptically inoculated with mold spores by adding 15 gm. of sugar containing approximately 1000 spores per gm.(prepared as previously described[5]) to 135 gm. of sugar in tightly corked Erlenmeyer flasks which were paraffined. The molasses was inoculated with a single scoopful of mold. Solid rubber stoppers, paraffined three times at short intervals, were later used. All flasks were incubated at room temperature (averaging about 28° to 30°C) for 4 months.

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*Read at the Philadelphia meeting of the American Chemical Society, September, 1919.

†The authors are indebted to Mr. S. Byall for his assistance with the chemical analyses.

TABLE 8.

Analyses of Inoculated Molasses (Blackstrap).

MOLD	S. P.	S. C.	R. S.	H_2O	Brix	DRY BASIS S. P.	DRY BASIS S. C.	M. R.	M.	Bacteria Molds per Gm.
Check	32.7	37.4	16.68	30.42	69.6	46.9	53.7	.44	—	683
Cladosporium	32.0	36.8	17.07	31.55	68.5	46.7	53.8	.46	—	692
Aspergillus flavus	31.5	37.1	16.68	31.14	68.9	45.8	53.9	.44	—	325
Aspergillus S. Bainier	26.8	32.0	19.75	30.84	69.2	38.7	46.3	.42	—	600
Penicillium	27.5	33.4	18.31	30.89	69.1	39.7	48.3	.43	—	675
Syncephalastrum	36.2	40.5	17.53	27.70	70.2	49.8	55.9	.43	—	5S, 40
Aspergillus niger	30.6	35.5	17.46	30.82	69.2	44.3	51.4	.45	—	2An 958

KEY TO TABLES 8-12—S. P., single polarization; S. C., sucrose Clerget; R. S., reducing sugars; P. E., probable error; H_2O, moisture; M. R., moisture ratio; M, mycelium; Bact., bacteria; N, negative evidence of inversion; P., positive evidence of inversion; A. n., Aspergillus niger; A. nid., Aspergillus nidulans; A. f., Aspergillus flavus; B. A., Aspergillus S. Bainier; Cl., Cladosporium; F., unknown sterile fungus; P., Penicillium expansum; S., Syncephalastrum; X, too numerous to count; Number only, in last column, refer to number of bacteria per gm.

TABLE 9.

Analyses of Inoculated Molasses (5/6 Blackstrap, 1/6 Syrup).

MOLD	S. P.	S. C.	R. S.	H_2O	Brix	DRY BASIS		M. R.	M.	Bact. Molds per gm.
						S. P.	S. C.			
Check	38.7	43.0	13.64	30.31	69.7	55.7	61.8	.49	—	700
Cladosporium	36.6	41.3	14.43	30.88	69.1	52.9	59.7	.48	—	500
Aspergillus flavus	37.4	42.1	13.91	31.04	69.0	54.3	61.0	.49	—	508
Aspergillus S. Bainier	32.3	38.3	16.33	31.24	68.8	46.7	55.7	.46	—	492
Penicillium	34.5	40.3	16.68	31.32	68.7	50.2	58.7	.48	—	492
Syncephalastrum	35.4	41.1	13.15	31.06	69.0	51.3	59.6	.48	—	433
Aspergillus niger	37.3	41.9	13.29	30.51	69.5	53.7	60.3	.49	—	441

TABLE 10.

Analyses of Inoculated Molasses (4/6 Blackstrap, 2/6 Syrup).

MOLD	S. P.	S. C.	R. S.	H_2O	Brix	DRY BASIS		M. R.	M.	Bact. Molds per gm.
						S. P.	S. C.			
Check	44.8	47.6	9.80	31.43	68.6	65.4	69.4	.57	—	350
Cladosporium	39.9	43.8	13.55	31.39	68.6	58.1	63.8	.52	—*	408
Aspergillus flavus	42.8	45.8	11.04	31.64	68.4	62.7	67.0	.55	—*	767
Aspergillus S. Bainier	36.2	41.4	15.01	31.48	68.5	52.7	60.5	.49	—*	692
Penicillium	34.5	40.2	15.36	31.81	68.2	50.7	58.9	.49	—	442
Syncephalastrum	43.9	47.0	11.19	31.39	68.6	64.0	68.6	.56	—*	475
Aspergillus niger	39.0	43.3	13.34	31.39	68.6	56.9	63.1	.52	—*	

TABLE 11.

Analyses of Inoculated Molasses (3/6 Blackstrap, 3/6 Syrup).

MOLD	S. P.	S. C.	R. S.	H_2O	Brix	DRY BASIS		M. R.	M.	Bact. Molds per gm.
						S. P.	S. C.			
Check	49.2	51.0	7.60	32.14	67.9	72.4	75.2	.63	—	600
Cladosporium	43.2	46.4	11.69	32.35	67.7	63.9	68.5	.57	—	733
Aspergillus flavus	41.2	45.0	10.16	31.52	68.5	60.3	65.9	.55	—	658
Aspergillus S. Bainier	39.0	43.1	13.95	29.81	70.2	56.2	61.5	.49	—	708
Penicillium	37.4	42.0	13.04	32.35	67.6	55.2	62.1	.52	—	683
Syncephalastrum	49.1	50.8	8.32	31.62	68.4	71.9	74.2	.62	—	450
Aspergillus niger	42.4	46.0	13.29	32.19	67.8	62.5	67.9	.56	—*	3An 567

TABLE 12.

Summary Showing Per Cent. Increase in Reducing Sugars in Inoculated Molasses After 4 months' Incubation.

Concentration	Cladosporium	Aspergillus flavus	Aspergillus S. Bainier	Penicillium expansum	Syncephalastrum	Aspergillus niger	Moisture Ratio
Blackstrap	—	—	18.4	0.8	—	4.7	.44
5/6 B. S. 1/6 Syrup	5.8		10.7	22.3			.49
4/6 B. S. 2/6 Syrup	38.3	12.3	53.2	56.7		36.1	.57
3/6 B. S. 3/6 Syrup	53.8	33.7	83.6	71.6	9.5	73.6	.63

In Tables 8-11 are the averages of triplicate determinations, and in Table 12 is presented the summary of the per cent. increase in reducing sugars over check as result of the inoculation of molasses by molds. It will be observed that with each mold used the per cent. increase in reducing sugars is made greater with a decrease in concentration of the molasses medium at the end of a 4 month incubation period. There was a corresponding decrease in sucrose Clerget in every sample. While this increase is striking, it does not follow a rigid mathematical progression, due to certain discrepancies which result from such a method of inoculation. It will be seen from Tables 8-12 that *Aspergillus Sydowi* Bainier and *Aspergillus niger* exhibit the maximum inversion, closely followed by *Penicillium expansum*. This corroborates previous conclusions concerning the deteriorative activity of these organisms both in the mycelial and spore stages (5,6).

Since such definite increases were obtained when molasses of known concentrations were used, it might be expected that refined sugar coated with such different molasses would yield similar results. That this is practically the case may be judged from the data in Table 13 and Table 14, which represents a summary of the per cent. increase in reducing sugars over check as resulting from an inoculation with mold spores at the rate of 100,000 per gm. after 1 months's incubation. It will be seen that an increase in moisture ratio:

TABLE 13.

Analyses of Inoculated Sugars with Films of Varying Concentration.

Concentration	Mold	S. Pol.	S. Clerg.	% Red. Sug.	Gain R. S.	P. E.	H_2O	DRY BASIS		Loss S. C.	M. R.
								S. P.	S. C.		
A	Check	98.5	98.6	0.418		0.013	0.31	98.8	98.9		0.206
Blackstrap	Asp.n.	98.1	98.1	0.545	.127	0.007	0.35	98.4	98.4	0.5	0.182
Film	B. Asp.	96.6	96.9	1.090	.672	0.060	0.46	97.0	97.3	1.6	0.134
	Pen.	97.6	98.1	0.880	.462	0.040	0.29	97.8	98.3	0.6	0.120
B	Check	98.7	98.8	0.265		0.003	0.19	98.8	99.0		0.144
5/6 B. S.	Asp.n.	98.5	98.6	0.468	.203	0.006	0.11	98.5	98.6	0.4	0.071
1/6 Syrup	B. Asp.	97.1	97.7	0.969	.704	0.024	0.30	97.3	98.0	1.0	0.106
	Pen.	97.9	98.2	0.718	.453	0.007	0.29	98.1	98.4	0.6	0.134
C	Check	98.8	98.6	0.197		0.000	0.09	98.8	98.7		0.072
4/6 B. S.	Asp.n.	98.4	98.4	0.357	.160	0.007	0.09	98.5	98.4	0.3	0.055
2/6 Syrup	B. Asp.	97.2	96.8	0.585	.388	0.006	0.32	97.5	97.1	1.6	0.115
	Pen.	97.8	98.3	0.552	.355	0.012	0.25	98.0	98.5	0.2	0.111
D	Check	98.9	98.8	0.218		0.005	0.21	99.1	99.0		0.192
3/6 B. S.	Asp.n.	98.1	98.3	0.457	.239	0.009	0.30	98.3	98.5	0.5	0.158
3/6 Syrup	B. Asp.	96.2	96.6	1.070	.852	0.030	0.31	96.5	96.9	2.1	0.082
	Pen.	96.9	97.4	0.689	.471	0.009	0.27	97.1	97.6	1.4	0.082
E	Check	98.5	98.7	0.231		0.007	0.22	98.6	98.8		0.144
3/6 B. S.	Asp.n.	98.1	98.2	0.441	.210	0.010	0.17	98.2	98.3	0.5	0.089
3/6 Syrup	B. Asp.	96.8	97.4	1.100	.869	0.024	0.15	96.9	97.5	1.3	0.048
	Pen.	97.0	97.3	0.766	.535	0.012	0.13	97.1	97.3	1.5	0.044

TABLE 14.

Summary Showing Per Cent. Increase in Reducing Sugars of Inoculated Sugars After One Month's Incubation.

Concentration	Aspergillus niger.	Aspergillus S. Bainier	Penicillium expansum	Moisture Ratio
Blackstrap	31.0	142.9	95.2	.20
4/6 B. S. and 2/6 Syrup	75.0	195.0	170.0	.08
5/6 B. S. and 1/6 Syrup	70.4	270.4	170.4	.14
3/6 B. S. and 3/6 Syrup	95.7	391.1	230.1	.18
Do.	104.6	404.5	218.2	.20

$\text{M. R.} = \dfrac{\text{Moisture}}{100\text{—Polarization}}$, which means a decrease in concentration, is responsible for raising the per cent. increase in reducing sugars in most instances, except where blackstrap films were employed. The latter phenomenon may be accounted for by the fact that the molasses film was relatively thin, due to too long prolonged purging. However, the other data, despite negligible discrepancies, are substantial proof of the direct effect of concentration on the deteriorative activity of molds. This has been proven to be a case of true inversion by the fact that an increase in reducing sugars has almost invariably been accompanied by a decrease in sucrose Clerget in the same sample. As in all previous work, *Aspergillus Sydowi* Bainier exhibits the greatest power of inversion.

If the results in Table 14 are compared with those presented in Table 12, it may be noted that there was a greater deteriorative activity revealed in the former case where the moisture ratio was actually lower. This would seem, upon the surface, to contradict the conclusion that a lowering of the concentration of the medium increases the deteriorative activities of molds. However, it must be remembered that the deteriorative activity of molds in any single medium is a resultant of two or more variables, i. e., concentration and amount of inoculum. We have shown in another paper (7) that an increase in amount of inoculum is responsible for an increase in inversion in any sugar solution of definite concentration. And there is found corroboration of this principle in manufactured sugars, for the inoculum used in Table 13 was approximately 100 times as large as that employed in Tables 8-11. Consequently, this experiment shows that a decrease in concentration causes increased deteriorative activity in mold-infected sugars, all other things being equal. Nevertheless, the effect of other variables cannot be overlooked. A similar experiment was repeated over a period of four months and the data gave similar evidence with moisture ratios varying from .08 to .30 as shown in Tables 15-18.

TABLE 15.

Analyses of Inoculated Sugars (Blackstrap Molasses Film)

FUNGUS	S. P.	S. C.	R. S.	H_2O	DRY BASIS S. P.	DRY BASIS S. C.	M. R.	M.	Bact. Molds per gm.
Check	98.5	98.4	.38	.28	98.9	98.8	.18	—	23
Cladosporium	98.6	98.4	.33	.34	98.9	98.7	.21	—	228
Aspergillus flavus	98.2	98.1	.41	.25	98.5	98.4	.16	—*	17
Aspergillus S. Bainier	97.5	97.5	.74	.32	97.8	97.8	.13	—*	2BA,6S, 20
Penicillium	97.9	97.9	.85	.36	98.3	98.2	.18	—*	5 P, 30
Syncephalastrum	98.4	98.2	.45	.34	98.7	98.6	.21	—	483
Aspergillus niger	97.8	97.8	.61	.29	98.1	98.1	.13	—*	57 An, 18

*Some mycelium of original inoculation present, but no evidences of multiplication.

TABLE 16.

Analyses of Inoculated Sugar (5/6 Blackstrap, 1/6 Syrup Film)

FUNGUS	S. P.	S. C.	R. S.	H_2O	DRY BASIS		M. R.	M.	Bacteria Molds per gm.
					S. P.	S. C.			
Check	98.4	98.4	.56	.36	98.7	98.8	.22	—	25
Cladosporium	98.9	98.8	.31	.28	99.2	99.0	.25	—	26 An. 7
Aspergillus flavus	98.5	98.3	.50	.35	98.8	98.6	.23	—*	32
Aspergillus S. Bainier	98.1	98.1	.69	.33	98.4	98.4	.17	—*	2BA,15S, 3
Penicillium	97.8	97.8	.77	.47	98.3	98.3	.22	—*	2P, 3F, 28
Syncephalastrum	98.4	98.3	.53	.34	98.7	98.6	.21	—	483
Aspergillus niger	98.4	98.3	.55	.30	98.6	98.6	.19	—*	375,An.

*Some mycelium of original inoculation present, but no evidences of multiplication.

TABLE 17.

Analyses of Inoculated Sugar (4/6 Blackstrap, 2/6 Syrup Film)

FUNGUS	S. P.	S. C.	R. S.	H_2O	DRY BASIS S. P.	DRY BASIS S. C.	M. R.	M.	Bacteria Molds per gm.
Check	98.5	98.5	.64	.40	98.9	98.9	.27	—	28
Cladosporium	98.9	98.9	.53	.37	99.3	99.1	.34	—	38
Aspergillus flavus	99.1	98.9	.36	.23	99.3	99.2	.25	—*	75 Af 20
Aspergillus S. Bainier	98.1	98.1	.71	.44	98.5	98.5	.23	—	2BA, 20S 7
Penicillium	98.3	98.5	.74	.40	98.7	98.8	.23	—*	5P 30
Syncephalastrum	99.1	99.0	.32	.20	99.3	99.2	.22	—	33 An, 518
Aspergillus niger	98.4	98.4	.46	.24	98.5	98.5	.15	—*	1000 An,

*Some mycelium of original inoculation present, but no evidences of multiplication.

TABLE 18.
Analyses of Inoculated Sugar (3/6 Blackstrap, 3/6 Syrup Film)

FUNGUS	S. P.	S. C.	R. S.	H_2O	DRY BASIS S. P.	DRY BASIS S. C.	M. R.	M.	Bacteria Molds per gm.
Check	98.5	99.0	.50	.43	98.6	99.1	.29	—	25
Cladosporium	98.7	98.8	.62	.28	99.1	99.1	.22	—	2 C1, 27
Aspergillus flavus	98.7	98.8	.63	.25	98.9	99.1	.19	—*	3 An, 33
Aspergillus S. Bainier	97.4	97.8	.65	.52	97.9	98.2	.20	—*	10S, 18
Penicillium	98.4	98.5	.70	.33	98.7	98.8	.20	—*	3P, 319
Syncephalastrum	98.2	98.2	.80	.30	98.5	98.5	.17	—	985
Aspergillus niger	98.3	98.3	.70	.43	98.8	98.7	.25	—	883 An, 3

*Some mycelium of original inoculation present, but no evidences of multiplication.

In Tables 19-21 are presented the results of an attempt to produce varying factors of safety in granulated and Cuban raw sugars by causing them to absorb moisture in the autoclave by the "humidor" method previously described. (5) The flasks were incubated at 30°C. for one month. As

TABLE 19.

Analyses of Inoculated Sugars (No Moisture Added)

MOLD	S. P.	S. C.	R. S.	H_2O	DRY BASIS S. P.	DRY BASIS S. C.	M. R.	M.	Bacteria Molds per gm.
Check	99.0	99.2	.18	.18	99.1	99.3	.18	—	23
Cladosporium	98.7	98.7	.30	.16	98.9	98.8	.12	—*	2 An, 42
Aspergillus flavus	98.2	98.2	.50	.29	98.4	98.4	.16	—*	700 Af, 7
Aspergillus S. Bainier	97.5	97.7	.66	.34	97.9	98.1	.14	—	2BA,5S,3An2
Penicillium	98.9	98.9	.18	.14	99.0	98.9	.13	—	83P, 350
Syncephalastrum	97.4	97.8	.83	.57	98.0	98.3	.22	—	43
Aspergillus niger	97.6	98.0	.75	.37	98.0	98.3	.15	—	1033 An.

*Some mycelium of original inoculation present, but no evidences of multiplication.

TABLE 20.

Plantation Granulated

Analyses of Inoculated Sugars (Moisture Added)

MOLD	S. P.	S. C.	R. S.	H_2O	DRY BASIS S. P.	DRY BASIS S. C.	M. R.	M.	Bacteria Molds per gm.
Check	98.7	98.7	.24	.27	99.0	99.0	.20	—	48
Cladosporium	98.6	98.5	.42	.23	98.8	98.7	.16	—*	38
Aspergillus flavus	98.4	98.3	.41	.39	98.7	98.6	.14	—*	318 Af, 5An
Aspergillus S. Bainier	97 6	97.6	.61	.54	98.1	98.1	.23	—	5S,3 An, 23
Penicillium	96.2	96.3	.97	1.07	97.2	97.3	.28	—*	2P, 3 An, 42
Syncephalastrum	98.4	98.4	.23	.27	98.7	98.6	.16	—	17 An, 267
Aspergillus niger	98.3	98.3	.50	.33	98.7	98.6	.20	—	1000 An.

*Some mycelium of original inoculation present, but no evidences of multiplication.

TABLE 21.

Analyses of Inoculated Cuban Raw Sugar.

MOLD	S. P.	S. C.	R. S.	H_2O	DRY BASIS S. P.	DRY BASIS S. C.	M. R.	M.	Bacteria Molds per gm.
Check	95.5	96.0	.89	1.26	96.7	97.2	.28	—	17
Aspergillus flavus	94.4	96.5	1.42	1.52	95.8	98.0	.26	—	2Af2f25An 12
Penicillium	91.8	92.6	1.92	2.47	94.1	94.9	.30	—	5 P, 20
Aspergillus niger	95.3	95.5	.95	1.06	96.4	96.5	.22	—	37
Check	95.5	95.6	.96	1.12	96.4	96.7	.24	—	3 An, 22
Aspergillus flavus	95.2	95.5	1.00	1.46	96.6	96.9	.30	—*	8Af,23An, 72
Penicillium	91.0	92.2	2.02	1.12	92.1	93.3	.12	—	42 P, 20
Aspergillus niger	95.2	95.6	1.08	1.23	96.4	96.5	.25	—	20 An, 32

*Some mycelium of original inoculation present, but no evidences of multiplication.

may be anticipated the moisture ratios obtained were of wide range. In the first series recorded in Table 19 where no moisture was added, the usual deterioration phenomena occur, namely, with a sufficient mold infection, an inversion is to be noted in most instances. The greatest deterioration occurred at a moisture ratio of .22 followed by *Aspergillus niger* at .15, which had the greatest number of molds per gm. In a subsequent section it will be shown that the factor of safety must be considered simultaneously with the degree of infection in order to arrive at a true understanding of deterioration. In Table 20, where the moisture ratios are somewhat higher due to the absorption of moisture as indicated. Again the greatest deterioration occurs with *Penicillium expansum* at the highest moisture ratio, namely, .28 with *Aspergillus Sydowi* Bainier following at .23, succeeded by *Aspergillus niger* at .20 with largest number of molds per gm. The same general conclusions may be drawn from Table 21 where Cuban raw sugar was employed.

TABLE 22.

ANALYSES OF MISCELLANEOUS SAMPLES.

PLANTATION GRANULATED.

MOLD	S. P.	S. C.	R. S. %	Gain R. S. %	H_2O	Dry Basis		M. R.
						S. P.	S. C.	
Check	99.0	99.2	0.35		0.16	99.1	99.3	0.16
Cladosporium	98.5	98.6	0.45	0.10	0.27	98.7	98.8	0.10
Aspergillus flavus	97.8	97.9	0.63	0.28	0.51	98.3	98.4	0.23
Asp. S. Bainier	92.8	94.0	3.13	2.78	1.25	92.9	94.1	0.17
Pen. expansum	92.9	94.3	4.17	3.82	0.45	93.3	94.7	0.06
Syncephalastrum	98.9	99.0	0.46	0.10	0.36	99.2	99.3	0.33
Aspergillus niger	95.0	95.8	2.08	1.73	1.05	95.9	96.8	0.21
REFINED.								
Check*	99.0	99.0	0.33	.0	0.15	99.1	99.1	0.15
Aspergillus niger	97.6	96.8	0.66	0.33	0.45	98.0	97.2	0.14
Pen. expansum	95.0	95.4	1.04	0.71	0.87	95.8	96.2	0.17
Asp. S. Bainier	92.0	92.8	1.25	0.92	1.05	92.9	93.7	0.13

*Approximate.

In Table 22 are presented some miscellaneous analyses of sugars heavily inoculated with molds which were used as inoculum in the preceding experiments. In the plantation granulated series it will be seen from the column marked "Gain in R. S.%", that *Aspergillus Sydowi* Bainer and *Penicillium expansum* followed by *Aspergillus niger* caused the greatest deterioration at comparatively low moisture ratios. There is a corresponding reduction in the sucrose Clerget which is in line with previously published results. It will be noted that *Syncephalastrum* and *Cladosporium* caused practically no deterioration in this incubation period of four months.

The series marked "refined" was one in which the sugars were liberally inoculated with spores to be used as inoculum in the succeeding experiment. The incubation period was one month and it is evident that a striking deterioration has taken place. The most active mold being again *Aspergillus Sydowi* Bainier followed by *Penicillium expansum* and *Aspergillus niger* in the order named. This confirms previous work and serves to substantiate further data.

Considering the importance of the moisture ratio in predicting the keeping quality of sugar in storage under what is known as the "factor of safety" rule, it is of special interest to note that here is presented evidence of the deterioration of manufactured cane sugars with moisture ratios from .08 to .09 when sufficiently infected with mold spores; and furthermore that this deterioration occurs even in films of the highest concentrations, namely, of blackstrap molasses. In sugar solutions we have already arrived at the resultant effect of the combined variables of concentration and amount of inoculum. In other words, in saturated sugar solutions upwards of 5000 spores per gm. are required to effect inversion. (7) We have further experiments which establish similiar criteria for sugars coated with films of known concentration as herein described. In this way, it is our purpose to arrive at a satisfactory method of judging the keeping quality of a sugar from the standpoint of mold infection, which we have show to be capable of inducing serious economic losses in sugar as a result of its deteriorative activity.

SUMMARY

1. A decrease in concentration of molasses inoculated with molds is responsible for a progressive increase in reducing sugars and a decrease in sucrose Clerget when incubated at 30°C for four months.

2. A decrease in concentration of laboratory-made sugars having films of known concentration and moisture ratios .08 to .20 inoculated with molds caused an increase in reducing sugars (and a decrease in sucrose Clerget) which gave evidence of active deterioration. These sugars were incubated at 30°C. for one month, and similar results followed a like incubation of four months.

3. *Aspergillus Sydowi* Banier, followed by *Aspergillus niger* and *Penicillium expansum*, in the order named, effected the greatest deterioration in both molasses and sugar.

4. There is evidence that an increase in inoculum is responsible for an increase in inversion at any definite concentration.

This investigation with laboratory-made sugars corroborates previous results obtained with sugar solutions.

Part IV.

EFFECT OF VARYING THE AMOUNT OF INOCULUM AND CONCENTRATION ON THE DETERIORATION OF SUGAR BY MOLDS*

Previously it was shown that a decrease in concentration of laboratory-made sugars having films of known concentration was responsible for an increase in deterioration when heavily inoculated with mold spores. The industrial application of this conclusion is determined by two important variable factors, namely, the concentration of the films surrounding the sugar crystals, and the degree of infection. Therefore, a further investigation of the influence of these factors was considered necessary, the results of which are discussed below. (9)

The method of procedure was identical with that outlined above except that the incubation period was five and one-half months instead of one month. A series of sugars with films of known composition were made in the laboratory by coating large crystals of sterilized sugar with sterilized blackstrap molasses and 60° Brix sugar syrup in definite proportions and purging in the centrifugal, a method previously employed with success. Blackstrap molasses, 5-6 blackstrap + 1-6 syrup, 4-6 blackstrap + 2-6 syrup and 3-6 blackstrap + 3-6 syrup when arranged in order of increasing moisture ratio are designated as Concentration A, B, C, and D, respectively. These sugars were inoculated with *Aspergillus niger*, *Aspergillus Sydowi* Bainier and *Penicillium expansum*, at the rate of 100, 1000, and 10,000 spores per gm. At the end of five and one-half months incubation at room temperature the contents of each flask were analyzed for sucrose by direct polarization and modified Clerget, reducing sugars and moisture. It has been already shown that the most satisfactory criterion of deterioration of sugar is the gain in per cent of reducing sugars. In order to summarize the results as briefly as possible, in

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*We are indebted to Messrs. D. F. Stanfill, Jr., and R. S. Hays for their assistance with the chemical analyses.

Table 26 are given the increase over check of the averages of closely agreeing triplicate determinations of reducing sugars, the data being presented in Tables 23-25. The abbreviation M. R. is for Moisture Ratio which value is derived as follows: $\text{M. R.} = \dfrac{\text{Moisture}}{100 - \text{Polarization.}}$ Asp. n. is the abbreviation for *Aspergillus niger,* while Asp. S. B. and Pen. represent *Aspergillus Sydowi* Bainier, and *Penicillium expansum.*

TABLE 23.

Analyses of Inoculated Sugars with Films of Varying Concentration. After 5½ months' Incubation.

100 SPORES PER GM.

Concentration	Mold	S. Pol.	S. Clerg.	Red. Sug. %	Gain R. S. %	P. E.	H_2O	Dry Basis		Loss S. C.	M. R.
								S. P.	S. C.		
A	Check	98.6	98.7	.32		.014	.19	98.8	98.9		.14
	Asp. n.	98.4	98.1	.27		.014	.15	98.5	98.3	.06	.09
	Asp. S.B.	97.9	98.3	.29		.016	.13	98.0	98.4	.05	.06
	Pen. exp.	98.2	98.2	.27		.000	.12	98.3	98.3	.06	.09
B	Check	98.7	99.3	.38		.005	.20	98.9	99.5		.16
	Asp. n.	98.5	98.6	.36		.002	.18	98.7	98.8	0.7	.12
	Asp. S.B.	97.9	98.0	.47	.09	.024	.17	98.1	98.1	1.4	.09
	Pen. exp.	99.0	99.0	.39	.01	.007	.18	99.1	99.1	0.4	.18
C	Check	99.2	99.2	.18		.005	.13	99.3	99.3		.18
	Asp. n.	99.1	99.4	.18		.012	.18	99.3	99.6		.27
	Asp. S.B.	98.9	98.8	.27	.09	.005	.11	99.0	98.9	.04	.10
	Pen. exp.	99.2	98.5	.25	.07	.010	.11	99.2	98.6	.07	.17
D	Check	99.0	99.1	.23		.010	.24	99.2	99.3		.24
	Asp. n.	99.0	99.2	.20		.020	.16	99.2	99.3		.16
	Asp. S.B.	99.2	99.2	.33	.10	.005	.09	99.3	99.3		.11
	Pen. exp.	99.1	99.3	.33	.10	.015	.16	99.3	99.5		.18

TABLE 24.

Analyses of Inoculated Sugars with Films of Varying Concentration. After 5½ months' Incubation.

1000 SPORES PER GM.

Concentration	Mold	S. Pol.	S. Clerg.	Red. Sug. %	Gain R. S. %	P. E.	H_2O	Dry Basis		Loss S. C.	M. R.
								S. P.	S. C.		
A	Check	98.6	98.7	.32		.014	.19	98.8	98.9		.14
	Asp. n.	98.8	99.1	.32		.007	.21	99.1	99.3		.19
	Asp. S.B.	99.0	99.2	.34	.02	.005	.13	99.1	99.4		.13
	Pen. exp.	98.9	98.8	.34	.02	.005	.07	99.0	98.9		.07
B	Check	98.7	99.3	.38		.005	.20	98.9	99.5		.16
	Asp. n.	98.7	98.5	.35		.010	.25	98.9	98.7	0.8	.19
	Asp. S.B.	98.5	98.2	.43	.05	.012	.18	98.7	98.3	1.2	.12
	Pen. exp.	98.7	98.9	.42	.04	.005	.16	98.9	99.1	0.4	.11
C	Check	99.2	99.2	.18		.005	.13	99.3	99.3		.18
	Asp. n.	98.8	98.8	.30	.12	.021	.12	98.9	98.9	0.4	.10
	Asp. S.B.	98.9	98.9	.40	.22	.010	.09	99.0	99.0	0.3	.09
	Pen. exp.	98.9	98.7	.35	.17	.002	.18	98.8	98.9	0.4	.16
D	Check	99.0	99.1	.23		.010	.24	99.2	99.3		.24
	Asp. n.	98.9	99.0	.27	.04	.005	.23	99.1	99.2	0.1	.21
	Asp. S.B.	98.7	98.8	.41	.18	.040	.25	98.9	99.0	0.3	.19
	Pen. exp.	98.7	98.8	.41	.18	.010	.27	99.0	99.1	0.2	.21

TABLE 25.

Analyses of Inoculated Sugars with Films of Varying Concentration. After 5½ months' Incubation.

10,000 SPORES PER GM.

Concentration	Mold	S. Pol.	S. Clerg.	Red. Sug. %	Gain R. S. %	P. E.	H_2O	Dry Basis		Loss S. C.	M. R.
								S. P.	S. C.		
A	Check	98.6	98.7	.32		.014	.19	98.8	98.9		.14
	Asp. n.	98.8	99.0	.36	.04	.021	.25	98.8	99.3		.21
	Asp. S.B.	98.6	98.7	.53	.21	.010	.30	98.9	99.0		.21
	Pen. exp.	98.6	98.8	.43	.11	.016	.23	99.0	99.0		.18
B	Check	98.7	99.3	.38		.005	.20	98.9	99.5		.16
	Asp. n.	98.4	98.7	.41	.03	.012	.29	98.7	99.0	0.5	.18
	Asp. S.B.	98.6	98.9	.50	.12	.000	.28	98.9	99.1	0.4	.21
	Pen. exp.	98.3	98.6	.49	.11	.016	.28	98.6	98.9	0.6	.17
C	Check	99.2	99.2	.18		.005	.13	99.3	99.3		.18
	Asp. n.	98.3	98.1	.45	.27	.046	.22	98.5	98.3	1.0	.14
	Asp. S.B.	98.3	98.3	.49	.31	.010	.17	98.5	98.5	0.8	.12
	Pen. exp.	98.5	98.5	.46	.28	.046	.17	98.7	98.7	0.6	.12
D	Check	99.0	99.1	.23		.010	.24	99.2	99.3		.24
	Asp. n.	98.7	98.5	.35	.12	.010	.20	98.9	98.7	0.6	.15
	Asp. S.B.	98.2	98.4	.50	.27	.065	.26	98.5	98.6	0.7	.15
	Pen. exp.	98.4	98.4	.51	.28	.010	.22	98.6	98.6	0.7	.14

TABLE 26.

Summary Showing the Influence of Amount of Mold Inoculum on the Deterioration of Sugars with Films of Known Concentration.

Increase in Per Cent. Reducing Sugars over Check.

No. of Spores per gm.	Concentration A. M. R.=.14			Concentration B. M. R.=.16			Concentration C. M. R.=.18			Concentration D. M. R.=.24		
	Asp. n.	Asp. S. B.	Pen.	Asp. n.	Asp. S. B.	Pen.	Asp. n.	Asp. S. B.	Pen.	Asp. n.	Asp. S. B	Pen.
100	—	—	—	—	.09	.01	—	.09	.07	—	.10	.10
1,000	—	.02	.02	—	.05	.04	.12	.22	.17	.04	.18	.18
10,000	.04	.21	.11	.03	.12	.11	.27	.31	.28	.12	.27	.28

It will be seen from Table 23 that in every instance (but one) an increase in the number of spores per gm. caused an increase in per cent of reducing sugars over check. This held true not only at every concentration employed, varying in moisture ratio from .14 to .24, but likewise for every organism used at any single concentration. This fact is very significant and indicates conclusively that an increase in amount of inoculation of mold spores at any definite concentration is responsible for an increase in deterioration of sugar. This corroborates our previous work where solutions varying from 10 to 70 per cent. were employed (7) as well as the results obtained in the experiment just concluded (8) where an inoculation of 100,000 spores per gm. at each of the above-mentioned concentrations was employed. A closer scrutiny of the results presented in Table 25 reveals the fact that the increase over check of reducing sugars with an inoculation of 100 spores per gm. is insignificant at practically all concentrations. The same is true of an inoculation of 1000 spores in the two higher concentrations, namely A and B. This is of practical importance in defining the limits at which deterioration occurs, since in plantation granulated sugars the moisture ratio may be said ordinarily to be below .18. It is generally considered that good Cuban raw sugar likewise should have its moisture ratio below .25-.33. Thus it might be inferred from the foregoing data that where the moisture ratio is below .18 mold infection of less than about 5000 spores per gm. would cause slight, if any, deterioration. As a rule we have rarely found sugars which had more than 250 mold spores per gm., although no quantitative survey has been carried out as exhaustively as might have been desirable.

When the two lower concentrations C and D are considered, we find that there is evidence of deteriorative activity with 100 spores per gm., while with more than 1000 spores the deterioration is quite appreciable. It would appear, therefore, that the safety of a sugar (from the standpoint of mold infection) having a moisture ratio .17-.18 would have to contain less than 100 mold spores per gm. On the other hand, a mold infection of more than 10,000 spores per gm. will cause a deterioration in sugars with moisture ratios varying from .14-.24. It may be noted in this connection that in the previous investigation mentioned (8) an inoculation of 100,000 spores per gm. was responsible in one month for a deterioration in sugars with moisture ratios varying from .08-.20.

An interesting fact which corroborates all our previous work with sugars as well as with solutions is that at any definite concentration and with an equal number of spores per gm. *Aspergillus Sydowi* Bainier is more effective than *Penicillium expansum* which in turn is more effective than *Aspergillus niger* in deteriorative activity.

A microscopic examination was made of all the sugars under consideration and no mold mycelium was found to have developed at the moisture ratios employed, namely below .25. In Table 27 we have advanced positive evidence in addition to this of negative character which indicates that

TABLE 27.

Summary Showing the Effect of the "Moisture Ratio" on the Development of Mold Mycelium.

Moisture Ratio = $\frac{\text{Moisture}}{100-\text{Polarization}}$	No. of Instances where no Mycelium present.	No. of Instances where Mycelium Present.	Doubtful.
.0	8	0	0
.01	2	0	0
.02	1	0	0
.03	6	0	0
.04	2	0	0
.05	7	0	0
.06	6	0	0
.07	12	0	0
.08	5	0	0
.09	9	0	0
.10	7	0	0
.11	10	0	0
.12	9	0	0
.13	12	0	0
.14	9	0	1
.15	13	0	0
.16	13	0	1
.17	5	0	0
.18	9	0	0
.19	11	0	1
.20	13	0	0
.21	14	0	0
.22	11	0	0
.23	14	0	1
.24	9	0	0
.25	8	0	0
.26	9	0	0
.27	6	0	0
.28	3	1	0
Total	243	1	4
.29	1	3	0
.30	2	3	0
.31	0	1	0
.32	0	1	0
.33	0	2	0
.34	0	2	0
.35	0	3	0
.36	0	1	0
.37	0	1	1
.38	0	0	1
.39	0	1	0
.60	0	1	0
Total	3	19	2

a moisture ratio above .28 is required before mold mycelium will be developed. This might explain why infected sugars with moisture ratios above .30 would deteriorate rapidly, i. e. mold mycelium secretes invertase as do the spores and each branch of mycelium may develop upwards of 300,000 spores. From the data in Table 27 which represents a summary of all inoculated sugars examined for mold mycelium, where the moisture content and polarization were determined it will be seen that in 248 instances where the moisture ratio was below .28, only once was mold mycelium detected. On the other hand, out of a total of 24 examinations of sugars with moisture ratios over .28, there were 19 instances, or practically 80 per cent, where mycelium was found. Thus the significant fact that the spores of molds in sugar will only germinate and produce mycelium at moisture ratios over .28 is of considerable practical value in the problem of sugar deterioration, especially in predicting the keeping quality of a sugar.

TABLE 28.

Summary Showing the Influence of Concentration of Film on the Deterioration of Sugars by Molds.

Concentration	Moisture Ratio	Per Cent Gain in Reducing Sugars Over Check.								
		100 Spores per Gm.			1000 Spores per Gm.			10,000 Spores per Gm.		
		Asp. n.	Asp. S. B.	Pen.	Asp. n.	Asp. S. B.	Pen.	Asp. n.	Asp. S. B.	Pen.
A.	.14	—	—	—	—	.02	.02	.04	.21	.11
B.	.16	—	.09	.01	—	.05	.04	.03	.12	.11
C.	.18	—	.09	.07	.12	.22	.17	.27	.31	.28
D.	.24	—	.10	.10	.04	.18	.18	.12	.27	.28

In Table 28 is presented a summary of results so arranged as to show the influence of concentration of film on the deterioration of sugars by molds. It must be stated at the outset, however, that where the increasing increments of moisture are so slight as in the present instance, and especially when dealing with the activity of micro-organisms over such a long incubation period, it is hardly to be expected that the differences will be very sharply defined. However, it will be observed that as a rule an increase in moisture ratio (which actually signifies a decrease in concentration) is responsible for an increase in deterioration with any single inoculation. It is not necessary here to repeat what was stated in the discussion of Table 26 concerning the limiting effects of concentration with any definite inoculum. Suffice it to say that this work fits in very closely with our preceding investigations and proves quite conclusively that with a high mold infection, deterioration takes place in sugars with moisture ratios below .14 and in the previous experiment at .08. This substantiates the claim previously made that: "The factor of safety for sugars well infected with fungi would appear to be lower than is generally supposed," and defines more clearly what such limits must be. In other words, knowing the number of molds present in any sugar it may be predicted (from the standpoint of mold infection alone) what deterioration may be expected in a storage period of about five months with a sugar of known moisture ratio. It is to be assumed that at the present time sugars are not stored for such a long period of time, but the differences obtained upon a long incubation period are not so much greater as to invalidate the above generalizations as will be seen from Table 29 which

TABLE 29

Comparison in Content of Reducing Sugars of Inoculated Sugars (with Films of Known Concentration D) after 1 and 5½ months Incubation Respectively.

INCUBATION	Check	Asp. n.	Gain over Check	Asp. S. Bainier	Gain over Check	Pen. exp.	Gain over Check
After 1 Month	.23	.44	.21	1.10	.87	.77	.54
After 5½ Months	.23	.53	.30	1.46	1.23	.87	.64
Gain R. S. %	.00	.09		.36		.10	

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gives a comparison in content of reducing sugars of inoculated sugars with films of known concentration D—(moisture ratio=.24) after one and five and one-half months respectively. It will be readily seen that the differences in incubation as represented by the gain in per cent of reducing sugars are indeed slight when compared with the initial gain over check in one month.

TABLE 30.

Deterioration to be Expected from a Definite Number of Molds in Sugars of Known Moisture Ratio.

$$\text{Moisture Ratio}=\frac{\text{Moisture}}{100\text{—Polarization}}$$

No. of Mold Spores Per Gm.	.08	.14	.16	.18	.20	.24	Over .24
0—100	—	—	—	+	+	+	+
100—1000	—	±	±	+	+	+	+
1000—10000	±	+	+	+	+	+	+
10000—100000	+	+	+	+	+	+	+

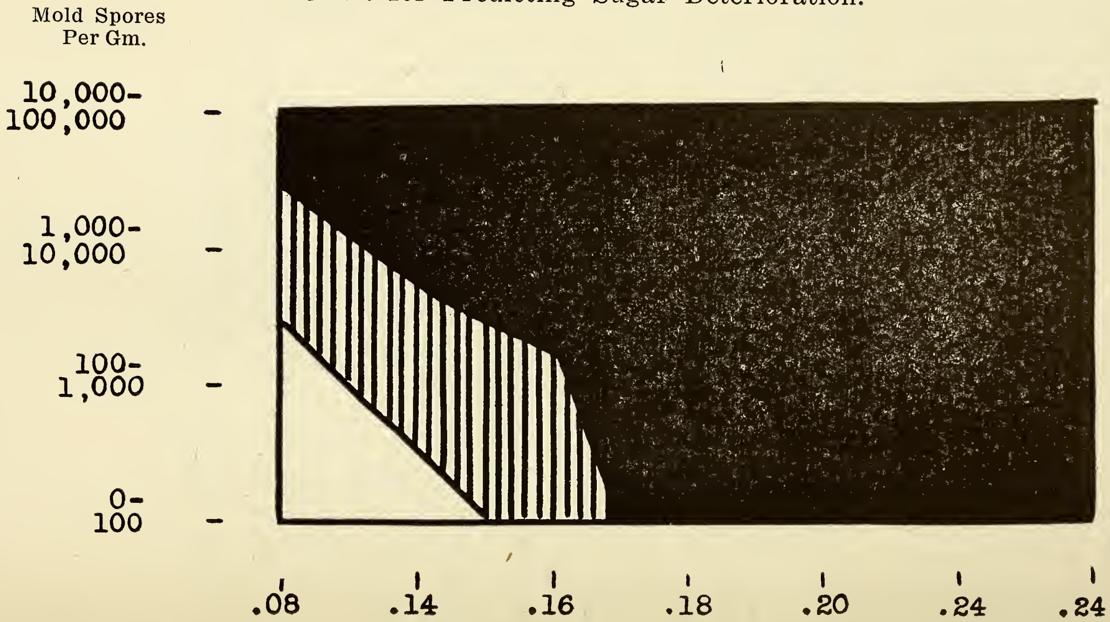
+ Deterioration.

— No Deterioration.

± Slight if any Deterioration.

Next to an elimination of deterioration, the most important commercial consideration is to predict the keeping quality of a sugar. Table 30 is a tentative plan based on the results obtained in all our investigations which will give some conception of the deterioration to be expected from a definite number of molds in sugars of known moisture ratio. It must be clearly understood that this plan is advanced with considerable diffidence, and that its value rests on further verification. Furthermore, it is of importance to note that in the above table only mold infection has been considered. We have data which are concerned with deterioration due to bacterial infection and unquestionably the bacterial flora would seriously influence the deterioration of sugars as shown in the important researches of previous investigators. However, since individual molds, such as *Aspergillus Sydowi* Bainier, *Penicillium expansum* and *Aspergillus niger* are vastly more efficient in their deteriorative activities than any bacterium that has come to our attention, and since the first named mold is to be found in practically all sugars, it may be that the above table will prove of some value to those who are ready to take cognizance of the molds which are undoubtedly causing large economic losses in the sugar industry. In Fig. 1, it will be seen that the facts previously discussed have been so arranged that one may tell at a glance what deterioration, if any, might be expected. It was not deemed necessary to carry out the work in moisture ratios beyond .24, because it is generally conceded that sugars having a moisture ratio above .30 are susceptible of deterioration. Browne (1), Owen (14) and others have advanced much valuable evidence on this point.

FIGURE 1.
Chart for Predicting Sugar Deterioration.



MOISTURE RATIO = $\frac{\text{MOISTURE}}{100\text{—POLARIZATION}}$

LEGEND—Heavy black, deterioration; Lined, slight, if any, deterioration; Colorless, no deterioration.

SUMMARY

1. An increase in number of mold spores inoculated into sugars (with films of varying concentrations) was responsible for an increase in deterioration.

2. A decrease in concentration of the films surrounding the sugar crystals was responsible for an increase in deterioration with a definite number of spores.

3. A table is presented showing the deterioration which may be expected from a definite number of molds in sugars of known moisture ratio.

4. At moisture ratios of less than .18 there is little, if any, deterioration with a mold infection of less than 5,000 spores per gm. More than this number of spores induces deterioration. At moisture ratios above .18, deterioration occurs with upwards of 100 spores per gm.

5. At any definite concentration and with an equal number of spores per gm. *Aspergillus Sydowi* Bainier is more effective than *Penicillium expansum* or *Aspergillus niger* in its deteriorative activity.

Part V.

THE PRESENT STATUS OF THE PROBLEM OF SUGAR DETERIORATION.

A retrospect over the investigations in sugar deterioration reveals the fact that considerable progress has been made towards a solution of the problem. The microbiological nature of the causative agents has been conclusively demonstrated; and valuable contributions concerned with the activities of bacteria have been fundamental in any approach to the problem. More recently we have indicated (5, 6, 7, 8, 9) the nature and deteriorative activities of the molds, which seem to be the most destructive group of microorganisms. Without losing sight of the major thesis it became necessary to investigate the deteriorative power of molds in the spore, as well as the mycelial stage. This led to the discovery that mold spores contained the enzyme invertase which was capable of deteriorating sugar solutions of high concentration. The number of spores per gm. proved to be a limiting factor in this connection. Applying these results to sugars of known concentration, it was found that the general conclusions previously arrived at were further substantiated. Thus was formed the basis for predicting the keeping quality of sugar, which depends upon moisture ratio or factor of safety and the number of microorganisms considered simultaneously, together with such environmental factors as temperature, humidity, etc. It was therefore possible to prepare a chart which would indicate at a glance what deterioration, if any, might be expected from a sugar of definite polarization and moisture content, infected with a known quantity of mold spores. Finally, in order to complete this phase of the problem, the importance of bacterial infection has been considered. In an experiment just completed (the results of which will be published in the near future) 320-pound bags of Cuban raw sugars were kept under normal storage conditions in the warehouse of one of the largest sugar refineries in this country, and analyzed periodically both chemically and bacteriologically. It was found that the data obtained in this manner were in close agreement with the investigations presented in this bulletin and indicated that deterioration is the resultant of the two factors operating simultaneously, namely, moisture ratio and infection, and that upon such knowledge it becomes possible to predict the keeping quality of sugars and thereby prevent serious economic losses.

We are cognizant of the fact that these investigations represent but a step towards the solution of the important problem of sugar deterioration, and that the practical deductions concerning cleanliness in and about the centrifugals, proper drying of the sugar, clean wash water, good storage, etc., arrived at in a previous bulletin (5) hereby receive additional emphasis. Proper precautions in handling invariably improve the keeping qualities of a sugar and should be practised by those interested in reducing losses of sucrose. But having studied the micro-organisms responsible for the inversion of sucrose, it is possible for us to proceed towards their elimination by methods now in progress.

In conclusion, the authors are appreciative of the assistance they have so generously received from the members of the station staff, Dr. C. A. Browne and Mr. W. L. Owen.

SUMMARY

1. A chart is presented which makes it possible to predict the keeping quality of sugar (from the standpoint of mold infection). This is based upon the following results.

2. Mold spores contain invertase which caused a decrease in polarization and an increase in reducing sugars in 10 and 20 per cent sterile sugar solutions.

3. The invertase activity of the spores of *Aspergillus Sydowi* Bainier, *Aspergillus niger* and *Penicillium expansum* is exhibited at concentrations of sugar varying from 10 to 70 per cent with a maximum activity at 50-60 per cent. An increase in the number of mold spores is responsible for increased invertase activity in a saturated sugar solution. The least number of spores per cc. of *Penicillium expansum*

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and *Aspergillus niger* required to produce inversion in saturated sugar solutions is between 50,000 and 100,000. Only about 5,000 spores of *Aspergillus Sydowi* Bainier are needed to cause inversion. The spores of this mold contain an enzyme capable of forming a gum in a sterile sugar solution.

4. A decrease in concentration of molasses inoculated with molds is responsible for a progressive increase in reducing sugars and a decrease in sucrose Clerget. This is likewise true of the films of laboratory-made sugars with moisture ratios of .08 to .20. *Aspergillus Sydowi* Bainier, followed by *Aspergillus niger* and *Penicillium expansum,* in the order named, effected the greatest deterioration in both molasses and sugar.

5. An increase in number of mold spores inoculated into sugars (with films of varying concentration) was responsible for an increase in deterioration. A decrease in concentration of the films surrounding the sugar crystals was responsible for an increase in deterioration, with a definite number of spores.

6. At moisture ratios of less than .18 there is little if any deterioration with a mold infection of less than 5,000 spores per gm. More than this number of spores induces deterioration. At moisture ratios above .18, deterioration occurs with upwards of 100 spores per gm. At any definite concentration and with an equal number of spores per gm. *Aspergillus Sydowi* Bainier is more effective than *Penicillium expansum* or *Aspergillus niger* in its deteriorative activity.

7. On the basis of the above results concerning the causative agents, elimination of sugar deterioration is now in progress.

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